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## U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF PLANT INDUSTRY-BULLETIN NO. 252.

B. T. GALLOWAY, Chief of Bureau.

# STUDIES OF FUNGOUS PARASITES BELONGING TO THE GENUS GLOMERELLA.

BY

C. L. SHEAR, Pathologist,

AND

ANNA K. WOOD, Formerly Scientific Assistant, Fruit-Disease Investigations.



WASHINGTON:
GOVERNMENT PRINTING OFFICE.
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## LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF PLANT INDUSTRY,
OFFICE OF THE CHIEF,
Washington, D. C., April 15, 1912.

SIR: I have the honor to transmit herewith and to recommend for publication as Bulletin No. 252 of the series of this Bureau the accompanying manuscript entitled "Studies of Fungous Parasites Belonging to the Genus Glomerella," by Dr. C. L. Shear, Pathologist in Charge of Grape and Small-Fruit Diseases, and Mrs. Anna K. Wood, formerly Scientific Assistant in the Office of Fruit-Disease Investigations. This paper has been submitted by Mr. M. B. Waite, Pathologist in Charge of the Office of Fruit-Disease Investigations.

This bulletin gives the results of studies of a group of fungous parasites of great economic importance. Few fruits are free from the attacks of this fungus. The life histories and relationships as well as the physiological and pathological characteristics of the organism from 36 different host plants are herein recorded, in many cases for the first time. It has been found that what had heretofore been regarded as distinct species of fungi restricted to certain host plants are in reality merely races or strains of one species which is capable of infecting various hosts.

These facts have a very direct and important bearing upon the practical problems of the prevention and control of the widespread and serious diseases caused by these parasites and also upon the broader general biological questions connected with the evolution of plant parasites.

Respectfully,

B. T. Galloway, Chief of Bureau.

Hon. James Wilson, Secretary of Agriculture.

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## STUDIES OF FUNGOUS PARASITES BELONGING TO THE GENUS GLOMERELLA.

#### INTRODUCTION.

The name Glomerella was first applied by Von Schrenk and Spaulding (70) <sup>1</sup> to the ascogenous form of the fungus producing the bitter-rot of the apple and the ripe-rot of the grape, usually referred to in its conidial condition as Gloeosporium or Colletotrichum. The same fungus, or fungi belonging to the same genus, attacks a great variety of other plants and produces diseases which are sometimes called "anthracnose," of which the anthracnose of the bean is a familiar example. In current usage, the term "anthracnose" is applied to diseases caused by fungi belonging to a few other genera besides Glomerella. It would be better if the name "anthracnose" were restricted to the disease caused by Glomerella. It still remains to be determined, however, whether some of the so-called anthracnoses are caused by Glomerella or not, as the complete life histories of the organisms are not all definitely known at present.

This genus of fungi is of vast economic as well as scientific importance. Few fruits are free from its attacks, and it is known to occur on a great variety of other hosts, from the palms to the highest flowering plants. It is also cosmopolitan in its distribution, though most

abundant in temperate and tropical regions.

All the facts connected with the life history of a parasite, the causes of its variability, its behavior under different conditions, and its relation to different hosts are essential to the most comprehensive and successful development of practical methods for the prevention and control of disease.

The primary objects of this investigation have been to determine the life histories and habits and the identity or relationship of the forms of Gloeosporium and Colletotrichum found upon the same hosts and on different hosts. Attention has also been given to the physiological features of the fungi and the possibility of their passage from one host to others. Careful studies of pure lines or races originating from single ascospores and single conidia have been carried on through many generations in order to determine, if possible, the conditions affecting the production of the ascogenous stage and the causes of the variations which sometimes appear in pure cultures.

<sup>1</sup> The numbers appearing in parentheses in this bulletin refer to the list of literature cited on pp. 101-105.

The first production of perithecia of Glomerella in pure cultures, so far as records have been found, was by Atkinson, as reported by Stoneman (89) in 1898. This ascogenous form was named Gnomoniopsis cingulata Stonem. (89). The conidial form had been described by Atkinson (2) as Gloeosporium cingulatum. Since Miss Stoneman's work was done considerable study has been given by different investigators to the life histories and relationships of Gloeosporium and Colletotrichum occurring upon different hosts. The systematic study and segregation of the species in this group of organisms has usually been based upon the supposed fixity of their host relationships and on some slight but inconstant differences in their morphological characters. A form occurring on a certain host was generally assumed to be distinct from one occurring upon another host, especially if there happened to be some slight difference in the measurements of the few spores observed or in the appearance of the affected portion of the host. Later, slight differences in the last two particulars led to the segregation in some cases of several species from the same host plant. Species were also sometimes based primarily upon the occurrence of the fungus upon different parts of the host. If the fungus at hand happened to occur upon the fruit it might be regarded as distinct from a form which was found upon the foliage or upon a shoot or branch of the same host. It is therefore necessary, before any satisfactory understanding or designation of these organisms can be obtained, to determine not only the ordinary range of variability of their morphological characters and their complete life histories, but also their host relationships. Where no fairly constant morphological characters can be found to separate the forms growing upon different portions of the same host plant, they should without question be referred to the same species. Where they occur upon different hosts, but still show no reasonably constant characters for identification and separation, they should still be regarded as one species. If it be held that these latter should be separated as so-called physiological species, the burden of proof falls upon those who take that position. Such a position can only be sustained by a sufficient number of successful cross-inoculation experiments to demonstrate that the organism will not pass from one host to another.

The present paper covers the investigation of members of this group of organisms obtained from 45 different host plants.

## PREVIOUS INVESTIGATIONS.

Previous work with these organisms may be divided into two parts: That primarily of a systematic or taxonomic character and that chiefly concerned with cultural, cross-inoculation, and life-history studies.

The conidial form of this genus of fungi is apparently much more common, conspicuous, and likely to be observed than the perithecial form. For this reason the majority of the species are much better known in their conidial condition than in any other and have been described principally under the names Gloeosporium and Colletotrichum, though some have apparently been referred to Cylindrosporium, Marsonia, and other similar genera. Four hundred and seventy-three species of Gloeosporium and Colletotrichum are given by Saccardo. This does not, as already suggested, include all of the species or forms that belong to this group, as some are found under other generic names. On the other hand not all the forms or species described under Gloeosporium and Colletotrichum are conidial stages of Glomerella.

It is quite certain, from a study of specimens and a comparison of the descriptions, that about 50 per cent of these so-called species can not be separated except on the basis of host relations or part of the host attacked. No monographic treatment of Gloeosporium and Colletotrichum has yet been attempted. The compilation of descriptions undertaken by Ellis and Everhart (33), in the Journal of Mycology, and that of Saccardo (67), in Sylloge Fungorum, are practically all we have.

Cross-inoculation experiments have been carried on at different times by different investigators. Owing to the various methods practiced in different cases and in some instances the lack of record of sufficiently definite information as to the details of the work, it is difficult satisfactorily to compare and coordinate the results. Southworth (84), Halsted (39, 40, 41), Cobb (20, 21), Clinton (19), Burrill (15), Edgerton (28, 29, 30, 31), Sheldon (77, 79), Chester (18), and Taubenhaus (90, 91) have made the principal contributions to this phase of the subject. A discussion of these results accompanied by tables will be found on subsequent pages.

Considerable attention has already been given by different investigators to cultural and life-history studies of these organisms. Among these may be mentioned the work of Southworth (84, 85), Atkinson (2), Stoneman (89), Clinton (19), Edgerton (28, 29, 30, 31, 32), Lasnier (55), von Schrenk and Spaulding (70), Sheldon (77, 78, 79, 80, 81), Shear (74), Barre (7, 8), Shear and Wood (75, 76), Koorders (54), and Scott (73). The work of Klebahn (51, 52) on Gloeosporium

relates to species which are not congeneric with Glomerella.

### PRESENT INVESTIGATION.

The present investigation was commenced by the senior writer in 1904. A brief summary of the early part of the work was published by the present writers (75) in 1907. At that time the life histories of the forms of Glomerella found on eight different hosts were briefly reported. During the same year a detailed description of the life history and behavior in cultures of Glomerella rufomaculans vaccinii Shear (74) was given by the senior writer in connection with a general discussion of cranberry diseases. In 1908 the present writers (76) presented a summary of their work before the Botanical Society of America, a brief abstract of which appeared in 1909. At that time the life histories of forms obtained from 18 different hosts had been worked out. Since then the number has been doubled, and both the conidial and ascogenous stages of Glomerella from 36 different hosts have been studied and the conidial forms from 45 different hosts have been grown, as follows:

GLOMERELLA CINGULATA (STONEM.) S. AND V. S.

## Conidia and perithecia produced either in culture or on the host:

Brya ebenus (L.) DC. (Jamaica ebony). Caryota rumphiana Mart. (palm).

Cinnamomum zeylanicum Nees (cinnamon).

Citrus aurantium sinensis L. (sweet orange).

Citrus decumana (L.) Murr. (pomelo).

Citrus limonum Risso (lemon). Citrus nobilis Lour. (mandarin).

Coffea arabica L. (coffee).

Costus speciosus (Koenig) Smith (spiral flag).

Curculigo sp.

Eriobotrya japonica (Thunb.) Lindl. (loquat).

Ficus carica L. (fig).

Ficus elastica Roxb. (rubber plant).

Ficus longifolia Schott.

Ginkgo biloba L.

Gleditsia triacanthos L. (honey locust).

## Conidia only produced:

Annona cherimola Miller (cherimoya). Crataegus sp. (hawthorn). Rubus trivialis (cult.) (white dewberry). Hedyscepe sp.=Kentia (palm). Ligustrum vulgare L. (privet).

Malus sylvestris Mill. (apple).

Mangifera sp. (mango).

Maranta arundinacea L.? (arrowroot).

Oxycoccus macrocarpus (Ait.) Pers.

(cranberry).

Persea gratissima Gaertn. f. (avocado).

Phormium tenax Forst.

Pimenta acris (Swartz) Kostel.

Piper macrophyllum Swartz (pepperwort).

Pitcairnia corallina Linden.

Psidium guajava L. (guava).

Ribes oxyacanthoides L. (gooseberry).

Rubus occidentalis L. (black raspberry).

Thea japonica (L.) Baill. (camellia).

Thea sinensis L. (tea).

Theobroma cacao L. (chocolate nut). Vitis labrusca L. (Concord grape).

Smilax medica Schl. and Cham. Vanilla planifolia Andrews (vanilla).

#### GLOMERELLA GOSSYPH EDGE.

Both conidia and perithecia produced in cultures: Gossypium hirsutum L. (cotton).

#### GLOMERELLA LINDEMUTHIANUM SHEAR.

Both conidia and perithecia produced in cultures: Phaseolus vulgaris L. (wax bean).

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GLOEOSPORIUM LAGENARIUM (PASS.) SACC. AND ROUM.

## Conidia only produced:

Citrullus vulgaris Schrad. (watermelon). | Cucurbita pepo L. (squash). Cucumis sativus L. (cucumber).

GLOEOSPORIUM MUSARUM CKE. AND MASSA

## Conidia only produced:

Musa paradisiaca sapientum (L.) Kuntz (banana).

#### METHODS OF STUDY.

#### DEVELOPMENT IN MOIST CHAMBER.

Apparently normal and healthy leaves, twigs, and fruits taken from plants showing a slight infection or suspected of being infected with anthracnose in a dormant or hibernating condition were found in most cases to develop typical spots of rot with acervuli and frequently perithecia when kept in a moist chamber. Such material was first immersed from 5 to 15 minutes in a 1 to 500 or 1 to 1,000 solution of corrosive sublimate to destroy any spores of the fungus which might be present. That this treatment is sufficient to destroy all the known reproductive bodies of Glomerella has been demonstrated by treating conidia, appressoria, and ascospores with these solutions for different periods. Treatment with a 1 to 1,000 solution of corrosive sublimate for three minutes has been found to kill all the spore forms.

After the foregoing treatment the specimens were rinsed in sterile water and placed in sterile glass moist chambers.

#### CULTURES.

Cultures have usually been started from conidia or ascospores obtained from fresh material from the field or greenhouse or from spores developed on parts of the host kept in a sterile moist chamber in the laboratory. To obtain pure cultures of the organism and to isolate single spores, poured plates of corn-meal agar have been generally used. Various solid or liquid media have been tried, but none has proved more satisfactory than corn-meal agar, which is prepared as follows: To four teaspoonfuls of corn meal add 1 liter of distilled water. Keep in water bath for one hour at a temperature below 60° C. Strain through gauze and to the filtrate add 1 per cent of agar flour. Steam three-quarters of an hour. Filter through filter paper. Tube and autoclave for 15 minutes at 115° C.

In isolating single spores to obtain pure-line cultures, special Petri dishes with very thin bottoms are used. After the plates have been poured and the agar cooled, the dishes are placed upside down on the microscope stage and single spores located with an objective of suffi-

cient power to distinguish and identify them clearly through the bottom of the dish. The location of the single spores is then indicated by a small circle of red ink on the bottom of the Petri dish. The culture medium in the dish should be very shallow, so that the range of the objective will reach through the full depth of the medium and permit the detection of spores lying at different levels. Some practice in getting the proper dilution of the spores in the medium is required. When the spores are too numerous in the plates it is frequently difficult to find one sufficiently isolated to be readily removed without the possibility of others being transferred with it. When the spores are too few, however, much time is required in searching over the plate to locate them.

Spores have also been isolated by the method described by Kauffmann (50). This method consists in sprinkling sterile water containing the spores on the surface of the agar after it has been poured in a Petri dish and cooled. When the medium has solidified, the spores are located with the microscope by examining the surface of the agar with the cover of the dish removed. This method has the advantage of insuring the distribution of the spores in practically one plane—i. e., on the surface of the medium—and permitting the use of a higher power objective than could be used when searching through the bottom of the dish. It has the disadvantage, however, of necessitating the removal of the cover during the search for the spores and thus greatly increasing the liability of contaminating the culture; but this tendency can be largely overcome by making the examination in a thoroughly protected culture room. With thin dishes and a thin layer of culture medium, spores not less than eight microns long can be located very satisfactorily by the first method. With smaller spores requiring high power for identification, the second method is preferable. As soon as the spores have germinated, which usually requires 8 to 16 hours, they are carefully transferred by means of flattened sterile needles to tubes of the same medium. If the germinating spore is transplanted near the upper margin of the agar and close to the wall of the tube, it may be usually located with the microscope and its actual transfer to the tube verified.

Pure lines, races, or strains can be isolated in this manner, then propagated indefinitely by transfer to subcultures or by the poured-plate method, thus making it possible to study the behavior of the organism in relation to various factors of nutriment and environment through as many generations as is desired.

## STUDIES OF GLOMERELLA FROM DIFFERENT HOSTS.

The following records give the results of the writers' studies of Glomerella, Gloeosporium, and Colletotrichum from various host plants, describing their behavior on leaves, stems, and fruits in

moist chamber and also in pure cultures. This matter is arranged alphabetically with reference to the host plants. Following the name of the host in each case is the name which has been adopted for the organism, with synonyms also where they have been satisfactorily determined.

## ANNONA CHERIMOLA MILL. (CHERIMOYA).

Gloeosporium rufomaculans (Berk.) Thüm.

Poured plates of conidia from an acervulus on a stem of cherimoya received from Miami, Fla., were made on April 9. Growth was rapid. The mycelium was white at first, changing later to dark greenish or smoke color and forming circular spots. Subcultures on corn-meal agar in tubes produced an abundant growth of mycelium and acervuli with a few setæ. Later many setæ appeared in these cultures. Though the cultures were kept growing until January 19 of the following year, no perithecia were ever found. The fungus from this host does not appear to have received a specific name. The characters of the acervuli and the shape and measurements of the spores averaged about the same as those from the apple, grape, and citrus fruits.

## BRYA EBENUS (L.) DC. (JAMAICA EBONY).

Glomerella cingulata (Stonem.) S. and v. S.

Several leaves of the host plant, collected in the greenhouse on January 19, were placed in sterile moist chamber. On February 8 acervuli of Gloeosporium were present on several of the leaves. On February 17 many mature perithecia were found associated with the acervuli. An ascus and ascospores are shown in Plate II, figures 23 and 23 a. The fungus showed no characters by which it could be distinguished from the Glomerella on apple and grape. No fungus of this kind appears to have been heretofore reported upon this host.

Plates were poured February 8, using conidia from a leaf taken from an apparently healthy plant in the Department greenhouse. The acervuli from which these cultures were made developed upon this leaf in a sterile moist chamber. Spores germinated quickly and subcultures in tubes of corn-meal agar were made by transferring single conidia. Growth in the plates was similar to that usually produced by Gloeosporium from apple or grape. Conidia were produced in both plates and tubes, but no very distinct acervuli were found. On February 17 the cultures were found to be contaminated with a mold and had to be discarded. Perithecia were not found in them. The cultures were perhaps not old enough at the time for perithecia to have had an opportunity to develop.

## CARYOTA RUMPHIANA MART. (PALM).

Glomerella cingulata (Stonem.) S. and v. S.

On January 29 leaves from a plant of this species, which was growing in the greenhouse and which had produced leaves with spots showing conidia of Glomerella, were placed in a sterile moist chamber. On February 3 a number of the leaves showed acervuli with conidia ranging from 13.5 to 25 by 4.5 to 6  $\mu$ . Setæ were present but not abundant. The conidiophores appeared to average a little shorter than is usual with forms from other hosts, being 9 to 15  $\mu$  in length. On February 18 many mature perithecia agreeing in practically all respects with Glomerella cingulata were found on these leaves. Asci and ascospores are shown in Plate II, figures 28 and 28  $\alpha$ .

The conidial form has apparently been described as *Gloeosporium* nanoti Prill. and Delacr. (64).

On February 7 cultures in corn-meal agar tubes were made by transfer of spores from acervuli on the leaves in moist chamber just described. Typical conidia and acervuli were formed in a few days. These cultures were kept until July 7, but no perithecia were ever found.

## CINNAMOMUM ZEYLANICUM NEES. (CINNAMON).

Glomerella cingulata (Stonem.) S. and v. S.

Leaves appearing perfectly healthy, taken from a greenhouse plant of this species, were placed in moist chamber. The leaves soon became discolored and several acervuli with conidia appeared. The conidial form agrees with Gloeosporium ochraceum Patterson (62).

On February 11 transfers of conidia were made from these acervuli to corn-meal agar tubes. These produced large acervuli and appressoria. Though kept until July 7 no perithecia were ever found. On April 30 more plates were poured from conidia from a leaf kept in moist chamber. A few peritheciumlike bodies were found in the plates on May 15. On May 23 some of these plates showed perithecia with mature asci. Subcultures to corn meal in flasks were made from the original tubes. These produced very few conidia but many sterile peritheciumlike bodies. Chlamydospores were also present. No asci were found in these cultures.

## CITRULLUS VULGARIS SCHRAD. (WATERMELON).

Gloeosporium lagenarium (Pass.) Sacc. and Roum.

On November 17, plates were poured, using conidia from an anthracnose spot on a watermelon grown at Vienna, Va. Transfers from plates to corn-meal agar tubes were made. Numerous acervuli soon appeared in these plates and very dark setæ were present in abundance. The setæ, however, were dark only at the apex and light colored at the base, which is not the case with the setæ in most other forms. They also appeared to be somewhat shorter than usual. No perithecia or peritheciumlike bodies appeared in the cultures.

The fungus agrees with Gloeosporium lagenarium (Pass.) Sacc. and Roum. It seems probable that the fungus occurring on cucurbits is specifically distinct from the other species investigated, though Halsted (40) reports the successful transfer of the organism from bean and pear to citron, Citrullus vulgaris var. He also reports the successful transfer of the organism from watermelon to bean, and as a result reduces Colletotrichum lindemuthianum to synonomy, using Colletotrichum lagenarium (Pass.) E. and H., which is the older name, for anthracnose of bean as well as that of cucurbits. Evans (36) states that the fungus passes from bean to watermelon and vice versa. but the statement is apparently based upon Halsted's work (39) and not on his own experiments. Further cultures and the opportunity to study the perithecial form from watermelon may be necessary to determine this point satisfactorily. The experiments of Edgerton (30) and the present writers in attempting to transfer the organism from bean to cucurbits, as reported later, were all failures. The writers' inoculations with the form from grape and guava to watermelon, however, were entirely successful, but not conclusive as to the identity of Colletotrichum lagenarium and Glomerella cingulata, since the perithecial stage of the former is unknown.

CITRUS AURANTIUM SINENSIS L. (SWEET ORANGE).

Glomerella cingulata (Stonem.) S. and v. S. Colletotrichum gloeosporioides Penz.

DEVELOPMENT ON LEAVES AND BRANCHES IN MOIST CHAMBER.

Since the fungus appeared to be present in a dormant or hibernating condition in the tissues of the leaves, as indicated by its appearance on apparently healthy leaves when their surfaces were thoroughly sterilized and placed in sterile moist chamber, several attempts were made to secure data which might throw some light upon the location of the fungus in the tissues and its original point of entrance. On March 6 apparently healthy leaves of various ages were taken from a blossoming tree in the greenhouse. These were treated as usual and placed in sterile moist chambers. On March 14 numerous acervuli were present on all the leaves except the youngest, which showed a number of discolored spots but no other external evidence of fungous infection. The acervuli on the older leaves usually appeared first at the base of the midrib, being preceded by a dark, water-soaked appearance of the tissue. In some cases the diseased

area gradually spread toward the tip until the entire leaf was affected, as illustrated in Plate VI. At other times acervuli broke out almost simultaneously on the entire surface and were equally numerous both on the upper and under sides of the leaf. On March 25 all but the present year's leaves, which were not entirely discolored, were covered with the fungus. Small patches of mature perithecia were also present. Though kept until April 13, the young leaves showed no trace of acervuli or perithecia of Glomerella.

On April 30 two more apparently healthy leaves were taken from near the tip of a shoot bearing fruit one-half inch in diameter. These were sterilized and placed in moist chamber, and two leaves from the previous season's growth on the same branch were treated in the same way. On May 7, accervali were abundant on the older leaves. On one, development was chiefly along the midrib; on the other, chiefly along the margins. The younger leaves showed no accervali. On May 11 one of the younger leaves showed discoloration and a few accerval at the base of the midrib. The other young leaf showed discoloration at the base, but had developed no accerval on May 13 when the leaves were discarded.

On May 11 a young sterile shoot—that is, a shoot without a fruiting bud—was taken from a greenhouse plant and the surface thoroughly sterilized as usual. It was then cut into six segments, each one bearing a single leaf. Each segment was placed in a separate sterile moist chamber. This experiment was performed to determine whether the fungus was equally abundant in fruiting and nonfruiting shoots; that is, whether the fungus might possibly enter the flower and gradually work back into the older parts of the shoot and foliage. These segments were numbered 1 to 6, beginning at the basal end. On May 18 all six leaves were more or less discolored, numbers 1 to 4 less than the others. In all cases the discoloration spread from the base of the leaf toward the apex. All but No. 2 bore acervuli. May 27 all the leaves were entirely discolored and almost covered with acervuli. The portions of the stem also showed acervuli. Since leaves 5 and 6, which were near the apical end of the shoot and youngest, became discolored slightly sooner than the older ones, it might, perhaps, be inferred either that they were infected earlier or that the tenderer tissues furnished more favorable conditions for rapid development.

On May 11 an apparently healthy fruiting shoot from the green-house was sterilized as usual and cut in segments which were placed in separate sterile Petri dishes. These segments were numbered 1 to 15, beginning at the basal portion of the shoot, as indicated in figure 1.

On May 18 the leaf on segment No. 1 was almost entirely covered with acervuli. The portion of the stem to which it was attached

showed no signs of the fungus. Segment No. 2 showed no indication of the fungus on either leaf or stem; No. 3 showed a slight uniform discoloration of leaf only; No. 4 showed discoloration of the leaf along the midrib and a very few acervuli. Three young fruits on this portion were also discolored and acervuli abundant on the calyx of one; also on the leaf petiole. No. 5 showed discoloration of the basal portion of the leaf and of the stem only. Nos. 6 and 7 showed leaf

and stem almost entirely discolored, but no acervuli. In No. 8 the basal portion of the leaf was discolored and a slight discoloration appeared on one side of the leaf, but no acervuli were present. No. 9 showed acervuli on the stem, but the leaf was not discolored. No. 10 had the entire leaf discolored. No. 11 showed the leaf almost wholly discolored, with the fruit black and bearing acervuli. On No. 12 the leaf and stem were almost entirely discolored, with acervuli at the base of the leaf. No. 13 showed the fruit completely discolored and acervuli present, but the leaf was not discolored. No. 14 showed the leaf entirely discolored, with acervuli on the cut surfaces of the stem. In No. 15 the leaf was almost entirely discolored, but no acervuli were present.

On May 20 leaves on Nos. 2 and 9 were still a normal color; all others, except 11 and 13, which were entirely discolored, showed abundant acervuli. Acervuli were present on all the fruits. On May 25 Nos. 2 and 9 had become discolored and showed abundant acervuli. Acervuli were also abundant on Nos. 11 and 13.

As a result of this experiment it will be noted that the fungus was present in all the leaves of the new growth as well as on the growth of the previous year. The fungus developed more rapidly on the older leaves. The early and vigorous development of the fungus in the young fruits and the leaves situated at their bases and the rather tardy development of the fungus in leaves remote from



Fig. 1.—An apparently healthy fruiting branch of an orange taken from a greenhouse plant, divided into 15 parts as indicated, then washed thoroughly in corrosivesublimate solution and kept in sterile moist chambers. Glomerella cingulata developed on all the leaves and fruit in such a manner as to indicate a probable origin from infection of the blossoms.

the blossoming shoots—that is, Nos. 2 and 9—seems to indicate a downward course of development of the fungus and infection by way of the blossoms, as shown by Rolfs (66) and Bessey (12).

CULTURES.

Numerous cultures have been made from conidia and ascospores obtained from orange leaves. Apparently healthy, vigorous orange

leaves taken from greenhouse plants have frequently been treated with corrosive sublimate and placed in sterile moist chambers. Such leaves have almost invariably produced an abundant growth of acervuli, which is frequently followed by the development of perithecia with typical asci and ascospores. The time required for the development of perithecia is usually two to four weeks.

Poured plates made with conidia from leaves kept in moist chamber produced a growth very similar to that obtained from other hosts. The number, size, and distribution of the acervuli formed vary considerably. Setæ were not usually present in these plates. Several series of plates produced only conidia. Two showed a few peritheciumlike bodies, but no asci were found. Different tubes showed considerable difference in the appearance of the growth. In some it was light colored and scanty; in others, abundant and dark.

Cultures were made from conidia taken from an orange leaf received from Houston, Tex., March 10, which was treated as usual and kept in sterile moist chamber until March 29. Transfers of single germinating conidia to corn-meal agar tubes were made. The growth was of a smoky-brown color, resembling that of cultures from pomelo hereafter described. A few large acervuli were formed, but no perithecia, though perithecia were produced on leaves in moist chamber. Later, setæ were found in abundance.

## CITRUS DECUMANA (POMELO).

Glomerella cingulata (Stonem.) S. and v. S. Colletotrichum gloeosporioides Penz.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On March 28 apparently healthy leaves of the pomelo were taken from greenhouse plants and placed in sterile moist chambers. They soon became spotted and acervuli appeared. These were soon followed on darker areas of the discolored leaf by perithecia, which showed mature asci on June 20. Setæ were not found in the acervuli on these specimens.

On April 30 two apparently healthy leaves were taken from immediately below a cluster of young fruit; also an older leaf, from whose axil a fruiting shoot was produced. These leaves were treated as usual and placed in sterile moist chambers. On May 7 acceptual were found regularly scattered over the surface of the older leaf, but there were none on the two young leaves. On May 13 the base of one of the young leaves showed slight discoloration running up the midrib three-fourths of an inch. On May 15 both young leaves were discolored along the midrib. Acceptual developed abundantly on these young leaves later. On June 9 perithecia and asci were found on the old leaf. This experiment, like those with the orange, apparently showed the presence of

the fungus in the tissues of both young and old leaves, but it is either more widely distributed in the old leaves or else develops in them more rapidly, as discoloration of the leaves and fructification of the fungus appeared sooner in most cases on old leaves.

On May 16 one of the young fruits about three-fourths of an inch in diameter was taken from the branch just above the two young leaves already referred to, the surface sterilized, and placed in a sterile moist chamber. On May 27 acervuli of Glomerella were found on the inside of the calyx and also on the fruit.

On March 6 other apparently healthy leaves, both young and old, were collected from a blossoming tree in the greenhouse, treated as usual, and placed in sterile moist chambers. Eight days later the 1-year-old and 2-year-old leaves showed abundant acervuli developing along the midrib, as indicated in Plate IV. The very young leaves in this instance showed no indication of the fungus. Ten days later the 1-year-old and 2-year-old leaves showed a number of discolored areas with perithecia. In this case the areas producing conidia and those producing perithecia were separate but contiguous, the two forms together almost covering the entire surfaces, as shown in Plate V. The young leaves now began to show discolored areas but no signs of fungous growth. On April 14 the youngest leaves were still free from any sign of fungus.

Other old leaves collected January 29 and treated as usual also developed an abundance of acervuli and perithecia.

#### CULTURES.

On March 16 cultures were made from conidia found on a fruit of pomelo received from Miami, Fla. Setæ were abundant in the acervuli on the fruit. The fungus grew rapidly, producing a smoky-colored growth like that in some of the cultures from the orange. Large acervuli were produced bearing a very few setæ. No perithecia were ever found in these cultures.

On May 14 other cultures were made from a pomelo fruit from Bonita, Cal. These cultures were made by transplanting portions of discolored spots or so-called "tear stains" from the fruit. The surface of the fruit was sterilized as usual with corrosive sublimate. The resulting growth was apparently pure and produced an abundance of acervuli and peritheciumlike bodies. These cultures were kept until August 13, but no asci or spores were found.

On June 17 corn-meal agar plates were poured, using conidia from acervuli surrounded by ascogenous perithecia from a leaf in moist chamber. These cultures all produced acervuli. They were kept until July 22 but showed no perithecia. No setæ or chlamydospores were seen in these cultures.

CULTURES FROM ASCOSPORES.

On June 25 poured plates were made with ascospores taken from a portion of a leaf in moist chamber which was producing perithecia only. A single ascospore was isolated and transferred to a slant agar tube.

On July 22 one plate showed a few perithecia with immature asci. No conidia were found. The tube culture from a single ascospore developed only a hyaline sterile mycelium and chlamydospores. Transfers from this tube to corn-meal flasks produced an abundant growth of white mycelium, which later became pinkish. On July 22 a very few spores, apparently conidia, were found. No typical acervuli or perithecia were produced in these cultures and it might possibly be suspected that the ascospores used did not really belong to Glomerella. They were, however, typical in appearance and morphological characters, and their identity could scarcely be doubted. The cultures from the ascospores on pomelo leaves did not produce, as is usually the case, many ascogenous fructifications. Only a few perithecia with spores were produced. Conidia were also scattered. No setæ were found, but chlamydospores were usually present. The ascospores from leaves in moist chamber varied from 18 to 23 by 4.5 to 6  $\mu$ . Asci were 60 by 10.5 to 12  $\mu$ . Paraphyses were found. The conidia from these cultures varied from 12 to 24 by 5 to 6 \(\mu\). Conidia from the leaves in moist chamber varied from 13.5 to 21 by 4.5 to 7 u.

## CITRUS LIMONUM RISSO. (LEMON).

Glomerella cingulata (Stonem.) S. and v. S.

DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

Leaves of lemon taken from a plant in the greenhouse and showing small discolored dead spots bearing a few conidia were placed in a sterilized moist chamber. An abundance of acervuli soon developed over both surfaces of the leaves. A few setæ were found. No perithecia were produced on these leaves.

On January 29 another leaf spotted in the same way and showing acervuli was placed in a moist chamber. On February 19 the leaf was almost covered with acervuli. A few setæ were present. A few areas bore perithecia agreeing in every respect with those found on leaves of other citrus species under similar conditions.

On January 29 three leaves apparently perfectly healthy were sterilized as usual and placed in a sterile moist chamber. On February 11 acervuli were present on the largest leaf, occurring mostly along the midrib and the larger veins. On February 15 this leaf had grown much darker and many acervuli were present. The other two leaves showed no signs of a fungus except that the petiole of one bore

a few acervuli. On April 7 abundant acervuli and perithecia had developed on all the leaves.

On April 30 two apparently healthy normal leaves were taken from a greenhouse plant just below an unopened flower bud, and a single leaf also was taken from the old wood lower down on the same branch. These leaves were sterilized as usual and placed in sterile moist chambers. On May 14 the old leaf showed discoloration extending from the base of the midrib to within half an inch of the tip, but no acervuli were present. On May 16 the end of the shoot from which the two leaves had been taken was cut off and placed in moist chamber. This shoot bore a young fruit about one-third of an inch in diameter, which had developed since the leaves were removed. The leaf next below these two was also taken and placed in moist chamber. On May 26 the old leaf showed acervuli. The two from below the unopened bud were still normal in appearance except for a slight discoloration at the base of the midrib extending up about three-fourths of an inch. The young fruit was also discolored, discoloration beginning at the stigma and working back. No acervuli or perithecia developed on the two young leaves and the fruit.

Since the foregoing was written, Essig (35) has recommended placing leaves in moist chamber to determine the presence of this fungus

in lemon groves.

#### CULTURES.

On December 12 two plates were poured, using conidia from a lemon leaf in moist chamber. On December 17 an abundant growth was present in the two plates, which differed greatly in appearance, though both appeared to be pure cultures. In one the mycelium was colorless and uniformly distributed over the surface showing many minute acervuli. Brown chlamydospores had also formed against the surface of the glass. No setæ were found. In the other plate the mycelium was slightly colored and denser, with dark points suggesting the beginnings of perithecia. No spores were yet found in this plate. On January 14 acervuli were thickly and uniformly distributed over the surface of the first plate and a few large acervuli were present in the second plate. Set were also found in the second plate but none in the first. No perithecia developed in either. Subcultures made from both plates to flasks of corn meal made a growth which was identical in appearance and produced an abundance of pink acervuli. No perithecia were found.

On December 18 four plates were poured, using conidia from four separate acervuli from the same leaf in moist chamber. Numerous acervuli developed in all, showing a tendency to form about cavities in the agar where portions had been removed for subcultures. This behavior was also shown in plates made from the orange, but was not noticed in the other plates from lemon.

On May 28 cultures were made in five flasks of corn meal by transplanting a portion of the discolored epidermis, the so-called "tearstain," of a lemon received from Bonita, Cal. The surface of the lemon had been sterilized by thorough washing with corrosive-sublimate solution. Appressoria or chlamydospores of Gloeosporium were found on the surface of the discolored skin. On June 2 the five flasks apparently contained pure cultures of Glomerella and were identical in appearance with those made in the same way from pomelo. Conidia were abundant, but no setæ were seen.

In discussing the diseases of the lemon caused by this fungus in California, Essig (35) states that the most satisfactory method of controlling them yet tried is by spraying with 4-4-50 Bordeaux mixture. This statement also accords with the results of Rolfs (66).

## CITRUS NOBILIS LOUR. (MANDARIN).

Glomerella cingulata (Stonem.) S. and v. S.

DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On January 29 four apparently healthy leaves from greenhouse plants were treated as usual and placed in a sterile moist chamber. On February 11 acervuli of Glomerella were present on all, having developed first at the base of the midrib. On February 29 a few perithecia with ascospores were found. Perithecia of the same Glomerella were also found on spotted leaves from the same plant, which had been kept in moist chamber.

#### CULTURES.

On March 20 two tube cultures were made from acervuli on the leaves in moist chamber. These cultures produced a growth of normal appearance, with acervuli but no perithecia. On October 7 two plates were poured, using conidia from a leaf in moist chamber. These plates produced an abundance of acervuli. A very few setæ were found. Setæ had also been found on the leaves from which the cultures were made but they were not numerous. Subcultures from these plates also produced an abundance of acervuli, but no setæ were seen and no perithecia ever developed, though perithecia with immature asci were found on the leaves from which the original cultures were made.

On November 14 subcultures on corn meal were made from the tubes just described. Many large acervuli soon formed. The spore masses frequently ran together and formed pink masses covering about one-half the surface of the medium. Dark spots resembling perithecia were also present but no asci or ascospores could be found. These cultures were kept until the following March, but no fertile perithecia were found and no setæ were observed.

It will be noted that perfect perithecia and asci of Glomerella were frequently produced upon leaves of the various citrus species when placed in a moist chamber. It is therefore somewhat remarkable that in only one case, that of the pomelo, were we able to get perithecia to develop in cultures.

The fungus from all the citrus hosts showed very similar characters in cultures and can not be distinguished from that on most other fruits. When old the mycelium is usually of a dark greenish or smoky color. The form of the fungus growing on citrus species has apparently been described several times under different names in both its conidial and ascogenous conditions. The conidial form has been treated by Rolfs (66) and others as Colletotrichum gloeosporioides Penz. (63). To judge from the descriptions and figures, it has also received several other generic and specific names. The conidia have been found to vary from 11 to 19.5 by 4.5 to 6  $\mu$ . The ascospores vary from 16 to 19 by 4.5 to 6  $\mu$ .

The perithecial form has apparently been described under several different names, including *Physalospora citricola* Penz. It is also found under *Laestadia socia* Penz.

### COFFEA ARABICA L. (COFFEE).

Glomerella cingulata (Stonem.) S. and v. S.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On December 4 leaves showing dead spots but no acervuli were taken from a coffee plant in the greenhouse. The surfaces were sterilized as usual and the leaves placed in a moist chamber. On December 30 acervuli and mature perithecia of Glomerella were found on these leaves. Setæ were present in the acervuli.

On January 29 two apparently healthy young leaves of the same age were treated in the same manner and placed in a sterilized moist chamber. On February 13 numerous acervuli were present on a discolored area, which extended downward from the tip of one of the leaves. The other leaf was entirely discolored but no acervuli were present. Later, perithecia of Glomerella were found associated with the acervuli on both leaves.

#### CULTURES.

On December 12 plates were poured using conidia from the leaves in moist chamber, on which perithecia developed later. A single conidium was located in one plate and transferred to a tube. On December 19 one plate had become contaminated and was discarded. The other contained a growth of the fungus of the usual appearance. Conidia were found. On December 26 acervuli were present in abundance in the tube and also in the plate. No setæ or perithecia were found. On January 8 two flasks of corn-meal agar were inocu-

lated by transfer of conidia from the tube culture from the single conidium mentioned above. On January 20 an abundant greenish growth was present, and many dark bodies resembling perithecia were forming. Many acervuli were also present. No setæ were found and no perithecia ever matured sufficiently to show asci and ascospores.

On June 12 two slant agar tubes were made by transfer from the single conidium culture. These tubes produced a growth of the usual appearance and small accrevili formed, but no perithecia were ever

found.

The growth and appearance of the conidial form from the coffee closely resembles that from other hosts, especially the Citrus species. Setæ and perithecia developed on the leaves in moist chamber but neither were found in the cultures made from the same leaves. The conidia from the leaves in moist chamber measured 13.5 to 16.5 by 5 to 6  $\mu$ . The conidia in culture varied from 15 to 18.5 by 4.5 to 6  $\mu$ . The ascospores from the leaves (Pl. I, figs. 14 and 14a) measured 15 to 18 by 5 to 6  $\mu$ . No paraphyses were found. The perithecial form found on the leaves suggested Laestadia coffeicola Speg. (86). The ascospores of this species are said to be obovate, and the measurements given are rather less than usual in Glomerella cingulata. Two species of Colletotrichum and two species of Gloeosporium have been described from coffee leaves. The descriptions of these species show no diagnostic characters sufficient to separate them satisfactorily. Gloeosporium coffeanum Delacr. (23) evidently refers to this species also.

COSTUS SPECIOSUS (KOENIG) SMITH (SPIRAL FLAG).

Glomerella cingulata (Stonem.) S. and v. S.

On January 30 portions of a leaf showing large dead areas were sterilized as usual and placed in a sterile moist chamber. On February 7 acervuli were found on these leaves, and on February 14 many mature perithecia of Glomerella were present. Asci and ascospores are shown in Plate II, figures 21 and 21a. No fungus of this kind in

either stage could be found reported on this host.

On February 7 two tube cultures were made by a transfer of conidia from the leaf in the moist chamber, each culture from a different acervulus. On February 24 one large acervulus was present in one tube and a few large sterile dark bodies in the other. On July 7 a few acervuli were present in the second. The peritheciumlike bodies were still sterile and no asci were ever found. The general appearance of the growth in the tubes was much like that of the fungus from other hosts but somewhat lighter colored. The conidia from the specimens in moist chamber showed the usual variations in shape, and they varied in measurement from 12 to 18 by 4.5 to 6  $\mu$ . The conidia in

cultures varied from 10 to 16.5 by 4.5  $\mu$ . The ascospores from the leaves in moist chamber showed the usual shape and varied from 15 to 21.5 by 5 to 6  $\mu$ .

#### CRATAEGUS SP.

Gloeosporium fructigenum Berk.

On December 12 three flasks of corn-meal agar were inoculated with spores from acervuli on a fruit of Crataegus which had been collected from a tree on the Department of Agriculture grounds and kept in moist chamber. The appearance of the acervuli and the decay of the fruit closely resembled that of the bitter rot of the apple. Conidia developed in these cultures and small dark bodies resembling perithecia were found in all the flasks, but no asci or ascospores were obtained.

## CUCUMIS SATIVUS L. (CUCUMBER).

Gloeosporium lagenarium (Pass.) Sacc. and Roum.

On June 20 four plates were poured, using conidia from a cucumber leaf collected at Portsmouth, Va. Two days later transfers were made to slant agar tubes. On July 6 the tubes showed an abundance of acervuli with a dense growth of brown setæ. The cultures became contaminated and no perithecia were formed. No opportunity was offered for a further study of this form and its identity with the others described is therefore uncertain.

#### CUCURBITA PEPO L. (SQUASH).

Gloeosporium lagenarium (Pass.) Sacc. and Roum.

On October 17 four plates were poured, using conidia from acervuli on a squash. The conidia and acervuli were of the same general appearance as those from other hosts. Setæ were abundant. On October 22 conidia were abundant in all the plates, but were scattered, no distinct acervuli being formed. The conidia were unusually variable in size, ranging from 8 to 30  $\mu$  in length. In the thickly sown plates dark bodies suggesting perithecia were abundant. None of these dark bodies were present in the thinly sown plates. These peritheciumlike bodies always remained sterile. Chlamydospores were also abundant. The mycelium was light colored. Later, many acervuli with pale pink masses of conidia developed in the tubes, and also an abundance of the sterile perithecia. The cultures were kept until January 13, but no ascospores were ever found in the perithecia. The writers' cross-inoculations of squash with the fungus from the grape were successful, but this does not necessarily prove that the organisms are the same. No inoculations from the squash to other fruits were made.

#### CURCULIGO SP.

Glomerella cingulata (Stonem.) S. and v. S.

On January 30 a leaf of this plant from the greenhouse, showing an elongated dead area in the center which bore acervuli of Gloeosporium and immature perithecia, was sterilized in the usual manner and placed in a moist chamber. On February 3 many dark spots had appeared on the apparently healthy portions of the leaf. Acervuli soon appeared in abundance, and later, setæwere found. On February 17 many mature perithecia of Glomerella were also present. Neither form of this fungus appears to have been heretofore reported on this host.

On February 7 two tube cultures were made by transfer of conidia from the leaf in moist chamber. Acervuli with pinkish masses of conidia soon developed, also chlamydosporelike bodies. No perithecia appeared, and subcultures kept until January 23 produced none. The conidia on the leaves varied from 14 to 16.5 by 4.5 to 6.75  $\mu$ . The ascospores measured from 12 to 24 by 5.5 to 6  $\mu$ . An ascus and ascospores are shown in Plate II, figures 22 and 22a. No characters could be found either on the host or in the cultures to distinguish this fungus from that occurring upon various other hosts.

## ERIOBOTRYA JAPONICA (THUNB.) LINDL. (LOQUAT).

Glomerella cingulata (Stonem.) S. and v. S.

On January 29 an apparently healthy leaf taken from a green-house plant which also showed some spotted leaves was treated as usual and placed in a moist chamber. On February 11 the leaf had turned dark brown and a few scattered acervuli were present. On April 8 acervuli were abundantly scattered over the surface. Setæ were sometimes present. No perithecia were found.

On the same date two spotted leaves from the same plant were placed in moist chamber. On February 12 abundant acervuli were present on these leaves. On February 20 perithecia of Glomerella were also found on the leaves.

Pure cultures made from acervuli from the leaves in moist chamber produced an abundance of typical acervuli, but no perithecia were ever found. The conidia from the leaves ranged from 12 to 18 by 4.5 to 6  $\mu$ ; the ascospores from 15 to 19.5 by 5 to 6  $\mu$ . Neither stage of this fungus seems to have been described from this host heretofore. Asci and ascospores are shown in Plate I, figures 11 and 11a.

## FICUS CARICA L. (FIG).

Glomerella cingulata (Stonem.) S. and v. S. Colletotrichum carica Stevens and Hall.

On April 23 plates were poured with conidia from the fruit of a fig received from Georgia. These plates produced conidia. The general appearance of the growth was very similar to that of the fungus from other hosts. Subcultures in tubes produced large scattered acervuli. Setæ were present but not numerous. No perithecia were found in these cultures.

On May 29 streak cultures were made on slant agar tubes, using conidia from acervuli on a decaying fig received from Norfolk, Va. On June 4 no acervuli were found, but a few perithecia with asci not quite mature had appeared. Later, mature asci were found in one tube. These asci were slightly shorter and broader than the average. Some of them contained only two or four spores. Normal asci and spores were also present, however. Other tube cultures developed later an abundance of typical perithecia. The asci showed a greater variability in size than usual on other hosts. (See Pl. II, figs. 29 and 29a.)

On July 3 cultures were made using conidia from a stem of the fig from Norfolk. An abundance of acervuli were produced in these cultures. Setæ were sometimes present and sometimes apparently wanting. Chlamydospores were also found. The conidial form is evidently Colletotrichum carica Stevens and Hall (88), and it can not be distinguished, so far as the descriptions go, from several other species which have been published. This fungus showed no characters, either in culture or on the host, which would serve to distinguish it from forms occurring upon other species of Ficus as well as those on other fruits, especially the apple. This identity is also confirmed by the cross-inoculation experiments with apples and grapes described later. Edgerton (31), who apparently studied the same organism, also states that it does not differ in any way from the form on the apple. As a result of numerous measurements of asci and ascospores, we find the asci range from 53 to 115 by 10.5 to 15 μ. The ascospores vary from 13 to 21 by 5 to 7  $\mu$ .

## FIGUS ELASTICA ROXB. (RUBBER PLANT).

Glomerella cingulata (Stonem.) S. and v. S. Neozimmermannia elasticae (Zimm.) Koord.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On January 14 apparently normal healthy leaves were taken from a greenhouse plant, treated as usual, and placed in a sterile moist chamber. On January 20 dark spots began to appear on the leaves. On February 14 the leaves were entirely discolored. No acervuli

were present, but a few mature perithecia of Glomerella were found.

Later, acervuli also developed.

On July 12 another apparently normal healthy leaf was treated in the same manner. On July 20 the under surface of the leaf had become light chocolate brown and was thickly covered with minute acervuli. Some larger, brighter colored ones were found on the petiole and about the midrib. No perithecia were found on this leaf. Many other leaves of this host have been treated in the same manner at different times, usually producing both conidia and ascospores.

#### CULTURES.

On February 11 streak agar tube cultures were made, using conidia from a leaf in moist chamber. On April 8 perithecia were abundant in one tube, being mostly aggregated in masses about the bases of old acervuli. Appressoria were also abundant on the surface of the glass in the upper part of the tube. Subcultures on corn meal in flasks produced an abundance of fertile perithecia also.

On April 1 bits of leaf bearing mature perithecia with ascospores were transferred to flasks of corn meal. On April 26 an abundant growth of conidia and also mature perithecia were found. Setæ

were sometimes found in the cultures but not regularly.

Koorders (54) has investigated this form of the fungus as it occurs in Java. He refers the perithecial form to a new genus, Neozimmermannia. There is, however, nothing in his description to separate his fungus from Glomerella and specimens of our plant submitted to Dr. Koorders for examination were said by him to be identical with his fungus. The conidial form has been called *Gloeosporium elasticae* Cke. and Mass. It can not be distinguished from the forms found on other *Ficus* spp. The asci were found to vary from 52 to 82.5 by 7.5 to 12  $\mu$ . Plate II, figures 18 and 18a, shows an ascus and ascospores.

#### FICUS LONGIFOLIA-SCHOTT.

Glomerella cingulata (Stonem.) S. and v. S.

## DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On November 18 two leaves of *Ficus longifolia* showing small dead areas were taken from a greenhouse plant, treated as usual, and placed in a sterile moist chamber. On December 6 numerous acervuli were present, producing masses of salmon-colored conidia. No setæ were found at this time. On January 4 setæ were present and also perithecia with mature asci and ascospores.

On January 29 another apparently healthy, normal leaf was taken from a greenhouse plant, sterilized as usual, and placed in moist chamber. On February 15 the leaf showed discolored spots of a dark-brown color and a few acervuli were found. Perithecia were also present, especially along the midrib. Some were single and others aggregated in groups. Mature asci were seen. The perithecia and ascospores were apparently identical with those obtained from other species of Ficus and could not be distinguished from the forms of Glomerella from various other hosts. Asci and ascospores are shown on Plate II, figures 19 and 19a.

#### CULTURES.

On December 12 plates were poured, using conidia from leaves in moist chamber. The growth was of the usual character and produced a few acervuli, but no perithecia ever appeared.

On February 3 subcultures from these plates were made in flasks of corn meal. The course of development was as usual, and on February 25 acervuli with pinkish masses of conidia were present. Peritheciumlike bodies were also found on the sides of the flask, but

no asci or ascospores could be discovered.

On April 28 plates of different dilutions were poured, using conidia from the cultures just described. On May 4 the plate most thinly sown showed a considerable number of acervuli. The next plate, in which more conidia were used, showed very few acervuli. The third plate, in which a still larger quantity of spores was used, showed many scattered conidia but no acervuli. In the cultures where numerous spores were sown, acervuli were much fewer than in those where the spores were more scattered. This behavior may perhaps have some direct relation to the greater quantity of nutriment available in the case of the thinner sowings than in the other. No perithecia were ever found in these cultures, although perithecia were present on the leaves from which the cultures were made. This may possibly have been due to the fact that both fertile and sterile strains were present on the leaves and the conidia used happened to be taken from acervuli belonging to a nonperithecium-producing strain.

Conidia from leaves in moist chamber varied from 15 to 20 by 5 to 6  $\mu$ , averaging slightly thicker than in most of the other forms. Conidia in plates varied from 13.5 to 16.5 by 5 to 6  $\mu$ . Ascospores ranged from 15 to 22 by 4.5 to 6  $\mu$ . No paraphyses were seen.

#### GINKGO BILOBA L.

Glomerella cingulata (Stonem.) S. and v. S.

Fallen leaves of this plant taken from the grounds of the Department of Agriculture were washed as usual and placed in a sterile moist chamber. In a short time acervuli of Gloeosporium formed on the leaves.

On November 13 potato-plug cultures were made by transferring conidia from the acervuli on the leaves in the moist chamber. On

November 23 an abundance of white mycelium was present in the cultures, also a number of black patches looking like areas of developing perithecia. On December 9 a flask of corn meal in which a subculture had been made from a potato plug showed an abundance of mature perithecia with asci and ascospores, which appeared identical with those of the Glomerella from other hosts.

On December 13 three plates were poured, using ascospores from the flask culture just described. On December 19 all the plates

showed perithecia and also conidia.

On January 4 other plates were poured from the same material apparently containing ascospores only. Conidia developed in a few

days, and perithecia also appeared later.

On January 23 subcultures were made in flasks of corn meal. On February 9 an abundance of perithecia and mature asci were present. Very few conidia were found. The fungus used in all these cultures appeared to belong to a race in which the perithecial form predominated. So far as known, no organism belonging to this group has heretofore been described or reported from the ginkgo. Plate II, figures 27 and 27a, shows an ascus and ascospores from leaves of this host.

## GLEDITSIA TRIACANTHOS L. (HONEY LOCUST).

Glomerella cingulata (Stonem.) S. and v. S.

On November 24 cultures in flasks of corn meal were made by transferring conidia from an acervulus on a leaf taken from a locust tree on the grounds of the Department of Agriculture. A little leaf tissue was also included in this transfer. On December 1 all of the cultures were identical in appearance and showed an abundant growth of young perithecia of Glomerella with asci but no fully mature ascospores. No acervuli or conidia were seen. On December 9 there was an abundance of mature perithecia and ascospores present. No distinct acervuli or conidia were positively identified in these cultures, though the cultures were derived from conidia or conidia-bearing mycelium from the leaf of the host. On January 4 poured plates were made, using ascospores from the flask cultures just mentioned. These ascospores germinated readily and produced a growth of the usual appearance. On January 8 conidia were found in these cultures. On January 10 two more plates were poured, using ascospores from the same cultures. These spores germinated, and on January 15 perithecia were found at many points in the plates. This strain also showed a great predominance of perithecia. In other respects it appeared identical with the Glomerella from other hosts. An ascus and ascopores from cultures from this host are shown in Plate II, figures 20 and 20a.

## GOSSYPIUM HIRSUTUM L. (COTTON).

Glomerella gossypii Edge. Colletotrichum gossypii South.

DEVELOPMENT ON BOLLS AND STEMS IN MOIST CHAMBER.

On February 20 leafless tips of cotton stems received from Lome, Togoland, western Africa, were treated as usual and placed in sterile moist chamber. These specimens showed dried whitish patches, suggesting old acervuli of Colletotrichum gossypii, but no conidia could be found. On February 23 acervuli with the characteristic brown setæ of this species were present on the stems. Transfers from these acervuli to flasks of corn meal produced an abundant growth of conidia and a dark mycelium but no perithecia.

On May 7 eight apparently healthy bolls about three-fourths grown were taken from a single plant in the greenhouse. The surface was sterilized as usual and the bolls placed in a moist chamber. On May 22 these bolls were more or less discolored, but no fungus was visible. On May 25 one of the largest bolls was almost completely covered with acervuli of *Colletotrichum gossypii*. On June 17 four other bolls also showed acervuli. As these bolls had been in the same chamber with the other, it is possible that infection came from the first boll.

On June 22 five other bolls of various ages were treated in the same way, but no Colletotrichum developed on any of them. These experiments would appear to indicate that the fungus on the cotton is able to live in a dormant or hibernating condition, as has been found to be the case with most of the other forms.

#### CULTURES.

On October 28, 1905, sections from a diseased cotton boll were transferred to flasks of corn meal. These all developed a growth resembling that of the cotton anthracnose, and on November 23 one flask showed acervuli with pink masses of conidia and also perithecia with ascospores not quite mature. All the other flasks showed a luxuriant growth of a white mycelium and conidia. Transfers were made from a flask producing perithecia. These also produced perithecia and ascospores.

Tests were also made of the effect of corrosive-sublimate solution, 1 to 1,000, on conidia of this fungus, the spores being treated from five to seven minutes and then transferred to poured plates. Check plates were made at the same time to determine the vitality of the spores. The checks grew luxuriantly, but no growth appeared in the plates sown with spores treated with corrosive sublimate, indicating that they had all been killed.

The appearance of this fungus in cultures is quite characteristic and remarkably uniform. The mycelium usually becomes dark colored rather early in its growth and the acervuli quite constantly produce an abundance of setæ which occasionally bear conidia, evidently indicating the derivation of the setæ from ordinary conidiophores. The production of the perithecial form of this fungus was first reported by the writers (75) in 1907 from the cultures described above. Later, Edgerton (29), 1909, reported finding the perithecial form on cotton bolls in Louisiana and named the fungus Glomerella gossypii. Since this fungus appears to possess certain morphological characters both under natural conditions and in cultures sufficient to separate it from its near relatives, it is apparently deserving of specific rank. Cross-inoculation experiments also seem to sustain this conclusion. An ascus and ascospores are shown in Plate I, figures 12 and 12a.

## HEDYSCEPE SP. (PALM)

Glomerella cingulata (Stonem.) S. and v. S.

Acervuli and perithecia with ascospores agreeing in all essential particulars with those of Glomerella as it occurs on other hosts were found on leaves of this host in the Department greenhouse. No pure cultures were obtained of this form. An ascus and ascospores from this material are shown in Plate II, figures 26 and 26a.

## LIGUSTRUM VULGARE L. (PRIVET).

Glomerella cingulata (Stonem.) S. and v. S. Gloeosporium cingulatum Atk.

DEVELOPMENT ON LEAVES AND STEMS IN MOIST CHAMBER.

Diseased leaves and stems of privet sent from Digby, Nova Scotia, and received September 30, showed fertile perithecia agreeing with the description of *Glomerella cingulata* except that the measurements of the ascospores were slightly less than those given by Stoneman (89). A single acervulus bearing conidia of the usual form and also showing a few setæ was also found on a sunken light-brown spot on one stem. This twig and others showing dark swollen points suggesting immature acervuli or perithecia were placed in a sterile moist chamber.

On October 4 mature perithecia had developed on this material and ascospores were oozing from the ostioles in light pinkish masses. Conidia developed a little later on the leaves and stems also. Perithecia also developed on the leaves in moist chamber.

CULTURES.

On October 16 plates were poured, using conidia from a leaf in the moist chamber. On October 24 many dark acervuli were present, the color being due in part to the dark basal hyphæ and in part to the brown setæ. On November 6 many mature perithecia were also found in the plates. Subcultures made by transfer of conidia from these plates also developed perithecia. Several other cultures made from the same material produced both acervuli with setæ and mature perithecia with ascospores. In some of the cultures the perithecial form predominated.

In order to study the behavior of the fungus with reference to its variability in different generations under practically the same conditions, a pure line or strain was isolated by taking single conidia from a plate which had also produced ascospores. These were transferred to tubes of corn-meal agar. The plates from which they were taken finally developed acervuli with setæ; also some perithecia. Only one of the tubes containing a single conidium gave a pure culture; the others became contaminated. This one produced several rather definite areas of mature perithecia and a few acervuli bearing pinkish masses of conidia. The second generation was obtained by transfers of conidia from this tube to two others. One of these tubes produced an abundance of perithecia, while the other contained fewer perithecia and a greater development of mycelium. Conidia were present in both, but were few and did not form in large, distinct acervuli. These cultures had the same general appearance as the first generations from ascospores described below.

On October 18 pure-line cultures were started, using a single ascospore from a poured plate made from ascospores derived from the original twigs. This culture developed a rather scanty growth of light-colored mycelium with but few conidia. Later, a few perithecia were found, but asci had not developed. The culture became contaminated with bacteria and was discarded.

One tube, to which a whole ascus had been transferred, produced a few inconspicuous acervuli with setæ and later an abundance of perithecia with mature asci and spores. Transfers were made from this tube, producing the second ascospore generation. A few conidia developed but no distinct acervuli. Mature perithecia and ascospores were also formed. The perithecia were not evenly distributed, as was sometimes the case in other cultures, especially those from the form on *Persea gratissima*.

Generation 3 was started by transfer of perithecia from generation 2. This culture produced an abundance of perithecia and a few conidia without acervuli. The appearance of the cultures was very similar

to that of generation 2.

For generation 4, six transfers were made of perithecia from generation 3. These soon developed numerous small acervuli with setæ. Few perithecia formed in these cultures. Other cultures made at the same time from ascospores from another series from this host made a growth of quite different appearance, no acervuli and very few conidia being formed. Few perithecia appeared. A further discussion of the pedigreed cultures will be given later.

Five transfers of single conidia were made from a plate subculture originally from a single ascospore culture. The growth produced in the five tubes was identical in appearance. A few acervuli developed near the point of inoculation and concentric rings of perithecia extended to the base of the culture. No asci were produced, however, so far as could be found. Plates poured from conidia from one of these tubes developed an abundance of conidia, but no distinct acervuli were formed. Chlamydospores and perithecia were present, but, as in the preceding generation, no asci developed.

The spores of this form, both ascospores and conidia, were very variable in size. The conidia in cultures ranged from 12 to 33 by 4.5 to 7  $\mu$ . On the host they ranged from 12 to 21 by 4.5 to 6  $\mu$ . Ascospores from the host ranged from 13.5 to 23 by 4.5 to 5.5  $\mu$ . Ascospores from cultures varied from 16.5 to 24 by 5 to 6  $\mu$ .

Miss Stoneman (89) gives the ascospore measurements of this species as 20 to 28 by 5 to 7  $\mu$ , which still further extends their range of variation. Unless the study of a vast number of spores from various localities should establish a constant mean spore measurement decidedly different from that found in Glomerella from other hosts, there seems to be no way of distinguishing this plant specifically from it. An ascus and ascospores are shown in Plate II, figures 17 and 17a.

## MALUS SYLVESTRIS MILL (APPLE).

Glomerella cingulata (Stonem.) S. and v. S. Glomerella rufomaculans (Berk.) S. and v. S. Gloeosporium fructigenum Berk.

Several attempts were made to develop this fungus from twigs of the 1-year-old growth taken from trees which had been badly affected with bitter-rot. In the 12 trials made no Gloeosporium was obtained in the cultures.

The production of the perithecial form from this host has already been described by several writers, both on the host and in pure cultures. It is therefore unnecessary to give a detailed account of our work on this point. Fertile perithecia of Glomerella are often produced on apples which have been attacked by bitter-rot; and they are also frequently obtained in cultures, though their production can not be depended upon in any particular strain. Here, as in forms from

other hosts, there appear to be fertile and sterile strains so far as the production of ascospores is concerned. Fertile perithecia have been produced in flasks of corn meal by transplanting portions of an apple taken from just beneath the skin where ascogenous perithecia were formed, and subcultures from these have continued to produce perithecia.

Leaves from trees badly affected with bitter-rot produced acervuli of Glomerella in abundance when kept in a sterile moist chamber. From this host, as from others, a strain of the fungus is sometimes obtained which produces peritheciumlike bodies in which no asco-

spores develop.

Fifteen tube cultures, made September 14, using conidia from acervuli on different bitter-rot spots on a Willow Twig apple from Vienna, Va., all developed perithecia but no acervuli. In five of the tubes the perithecia were fertile, producing asci and ascospores. The other 10 produced only immature or sterile perithecia. inoculated with ascospores from these cultures developed bitter-rot, followed by the production of fertile perithecia but no acervuli, indicating in this strain the great predominance of the perithecial form. Cultures made from apple leaves from West Virginia, obtained from Mr. Rand, produced perithecia with asci somewhat smaller than usual, as shown in Plate III, figure 39. Cultures of this form grown on sterile apple twigs also showed a few brownish ascospores.

The spore measurements of Glomerella as found on this host and in cultures are as follows: Asci from fruit in moist chamber 69 to 78 by 6 to 10.5  $\mu$ , in cultures 54 to 110 by 7.5 to 13  $\mu$ ; ascospores from apple 15 to 21 by 4.5 to 6  $\mu$ ; ascospores in cultures 13.5 to 21 by 4.5 to 6  $\mu$ . Variations in the asci from this host are shown in Plate III, figures 35 to 39. Plate I, figures 3 and 3a, also shows an ascus

and ascospores of the same.

#### CHROMOGENIC FORM.

On November 16 four plates of corn-meal agar were poured from a single acervulus of Glomerella from an apple from Vienna, Va., which had been kept in moist chamber in the laboratory. These cultures produced an abundance of acervuli and chlamydospores. In the dilute plates the acervuli were numerous; in the thickly sown plates acervuli were almost entirely wanting and the conidia scattered. These cultures when about a week old began to take on a pinkish color, the color apparently being developed in the agar and not in the mycelium of the fungus. Four tubes inoculated by transfer of spores from four different acervuli from this same plate were made to determine whether this chromogenic character was possessed by other acervuli and whether it persisted. Four days later a slight pinkish

tinge was visible in all the cultures, and at the end of a week the agar had become a decided pink color, and acervuli were present in all the tubes.

On November 24 three more plates and three tubes were made from an acervulus on the same apple used in the previous cultures. From the poured plates six transfers of single germinated conidia were made to tubes.

On November 28 three cultures made by direct transfer from an acervulus on the same apple showed the same pink color observed in all previous cultures from this apple. Acervuli were present. There was no color in the plates at this time, though acervuli were present.

On November 30 the pink color was very conspicuous in the six single-spore cultures, and the cultures were practically identical in appearance with those in the other tubes. The appearance of the pink color in all the pure single-spore cultures completely dispelled the natural suspicion that the first cultures might have been contaminated. These cultures, though kept for several months, never developed any dark mycelium, the growth of hyphæ being white or only slightly grayish. The cultures differed in this respect from most other cultures from apples and other hosts, which usually produce more or less of a dark greenish or smoky-colored mycelium, especially when old. Chlamydospores were present in these cultures, but differed slightly from the usual form, many being large and regular. In the following June transfers were made from these old cultures, which were still living, to slant agar tubes. The growth was very slow. On September 4 conidia were present, and the agar showed a slight pinkish color. One tube showed little or no color. Other transfers from a tube showing color made a more abundant growth of acervuli and showed more of the pinkish color.

On December 4 two sound apples were inoculated by puncture, using conidia from one of the single-spore cultures from this chromogenic form. The surface of the apples was carefully sterilized by washing as usual, after which they were placed in a sterile moist chamber. Decay began at the points of inoculation in a few days, and on December 17 the decayed areas on both of the apples were more than an inch in diameter and slight pustules, apparently young acervuli, were developing on the skin. On December 26 acervuli having all the characteristics of Glomerclla were numerous on the decayed spots. The conidia were extremely variable. No setæ were found.

On January 4 and 5 plates were poured, using conidia from one of the inoculated apples just described. Four transfers were made of single germinating spores to tubes of agar. The general appearance and development of the fungus in these tubes was very similar to that in the previous cultures of this chromogenic form. One culture failed to grow; the other three soon began to show the pink color in the medium. The color in these cultures never became quite so pronounced as in some others.

On January 6 six tube cultures were made by transfer of conidia from a single acervulus in one of the single-spore cultures just described. Three were on prune agar and three on a synthetic medium. The cultures on prune agar made rapid growth, produced an abundance of acervuli, and the medium became a decided pink color. No perithecia or peritheciumlike bodies ever appeared in any of these cultures. The constant white or gravish color of the mycelium, the chromogenic character of the fungus, and the variation in the conidia suggested very strongly the possibility that we were dealing with an organism different from the common bitter-rot fungus of the apple. The conidia were very variable in size. Those which were scattered were rather smaller than usual, and those borne in the acervuli were large and more uniformly pointed at the ends. As no perithecial form was found on the fruit from which the cultures were obtained nor in any of the cultures, it is possible that this may represent a distinct species or variety.

#### EUROPEAN FORM.

Schneider-Orelli (69) has recently reported the results of comparative studies of the conidial stage of Glomerella cingulata sent from Virginia and Gloeosporium fructiginum Berk. found on apples in Switzerland. He finds very little difference in the morphological characters of the American and European forms. A comparison of their physiological characteristics showed a difference of 5 degrees centigrade for minimum, maximum, and optimum growth. The minimum temperature at which growth occurred in the European material was 5°, the optimum temperature 23°, and the maximum temperature 27° C., while the minimum, optimum, and maximum for the American material was found to be 5 degrees higher, or 10°, 27°, and 32° C., respectively. Whether this difference would hold good with other races and strains from the two countries is a question. The writers have carried out no such comparative studies but have reason to suspect that further work in this direction might show that this physiological character is also variable in both the American and European forms. Little difference was found in the production of rot caused in stored apples when inoculated with the two forms. According to the writer just cited, as well as other European pathologists, the bitter-rot fungus is not common in Europe and where present causes very little loss. It seems probable that this is due, in great part at least, to the less favorable climatic conditions prevailing in parts of Europe where apples are mostly grown, the low average maximum summer temperature being perhaps the chief factor as compared with the average summer temperatures in the parts of the United States where the disease is most serious. As has been pointed out elsewhere in this paper, there is evidence to show considerable difference in the virility of different strains of the fungus from different sources in this country, and this fact may explain the difference in destructiveness of the disease in localities where climatic conditions are apparently equally favorable and the disease is present.

## MANGIFERA SP. (MANGO).

Glomerella cingulata (Stonem.) S. and v. S.

On May 6 leaves and stems of mango received from Hallandale, Fla., showing acervuli of Glocosporium, were placed in a moist chamber. On May 18 perithecia with immature asci and numerous acervuli were found on the leaves, and many acervuli were also present on the stems. In the absence of mature ascospores there is a possiblity that the perithecial form associated with the conidia was not Glomerella, but this is not probable.

On May 29 plates were poured, using conidia from the mango leaves in moist chambers, and later, single germinating conidia were transferred to tubes. These cultures produced an abundance of large acervuli with pink spore masses, and a few setæ were found. The general characters of the growth were as in the forms from other hosts, though the mycelium varied somewhat in quantity and color. No perithecia ever occurred in these cultures, though immature perithecia developed on the leaves from which the cultures were made. The conidia from a single acervulus in one of these cultures varied from 12 to 25.5 by 3.5 to 6  $\mu$ .

Three species of Gloeosporium have been described from the mango, G. mangae Noack (61), G. mangiferae Henn. (46), and G. raciborski Henn. So far as the descriptions go they can not be satisfactorily separated. The extremes in the spore measurements given for these three species are nearly all included within the range of the measurements found in the single acervulus just referred to, while setæ were present in some acervuli and wanting in others.

## MARANTA ARUNDINACEA L. (ARROWROOT).

Glomerella cingulata (Stonem.) S. and v. S.

On January 29 a portion of a leaf of this species, showing a small dead area, was taken from a greenhouse plant, treated as usual, and placed in a sterile moist chamber. On February 7 acervuli had developed, and a little later immature perithecia were found. Tube cultures were made by transfer of conidia from the leaf just described. These cultures produced a growth of the usual appearance of Glomerella. Numerous acervuli with pinkish masses of conidia were

formed. Though the cultures were kept for nearly a year, no perithecia were ever found in them. The fungus agreed in all essential particulars with *Glomerella cingulata*.

MUSA PARADISIACA SAPIENTUM (L.) KUNTZ. (BANANA).

Gloeosporium musarum Cke. and Mass.

Cultures of this fungus, which is frequently found upon decaying bananas, have been made at different times. The growth and appearance of the fungus in cultures in most cases is somewhat different from that of the other forms grown. A slight amount of white mycelium generally appears at first and soon becomes dotted, with small, dark bodies which suggest perithecia, but when carefully examined they prove to be small acervuli. The acervuli usually develop in great numbers and, being crowded, produce large continuous masses of salmon-pink spores. Chlamydospores were very abundant in some cultures. No setæ were ever found. A few dark perithecium-like bodies were occasionally found in old cultures, but no asci or spores ever developed.

Spore measurements obtained from conidia in a single acervulus in a culture were 9 to 28 by 4.5 to 6.5  $\mu$ . In other cultures conidia were found reaching 34  $\mu$  in length and 8.5  $\mu$  in thickness. With no knowledge of the perithecial stage of this form, it is not possible to make any positive statements in regard to its identity with the species of Glomerella from other hosts. In some cultures it showed essentially the same characters and appearance as in the forms from other hosts, while in others, as mentioned above, it showed rather different characters.

Most of the attempts to transfer the Gloeosporium on banana to other fruits have been failures. Laubert (56) had no success in inoculations from the banana to apples, though Cobb (21) reports success in producing bitter-rot of pears and quinces with the banana fungus.

OXYCOCCUS MACROCARPUS (AIT.) PERS. (CRANBERRY).

Glomerella cingulata vaccinii Shear.

This fungus has been found at various times on both fruit and foliage of the cranberry and has already been reported on by the senior writer (74). More recently the perithecial form has been again obtained in a number of cases from fruit collected in Massachusetts. Cross-inoculation experiments with apples have given negative or uncertain results. In one case a small decayed spot appeared, and a few acervuli formed. It is possible that this variety would adapt itself to another host in a few generations as some of the other forms have done.

## PERSEA GRATISSIMA GAERTN. F. (AVOCADO).

Glomerella cingulata (Stonem.) S. and v. S.

DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On January 15 apparently healthy leaves from a greenhouse plant with the surfaces sterilized as usual were placed in a sterile moist chamber. Dark spots soon appeared on these leaves, and on February 3 an abundance of acervuli with pinkish masses of conidia were present. No perithecia were found.

On July 1 other leaves of normal appearance from the greenhouse were treated in the same manner as the preceding. On July 26 numerous acervuli were present and also perithecia with immature

asci. Later, mature asci and spores were found.

On December 30 leaves showing small dead areas were taken from the same source and treated in the same manner as above. On January 7 abundant acervuli with setæ were found on one of the leaves. On January 17 a few mature perithecia were found on the same leaf. They were characterized by unusually long beaks. Later, acervuli and perithecia developed on the other leaf also.

#### CULTURES.

On December 18 five plates were poured, using conidia from a single acervulus on avocado fruit received from Miami, Fla. A few setæ were present in this acervulus. Three single germinating conidia were transferred to tubes. On January 5 both plates and tubes differed in appearance from the earlier cultures from Persea, especially in the scarcity, or almost complete absence, of conidia. A few dark stromatic bodies were present in the plates, but no perithecia. A very few acervuli finally developed in the tubes, but no setæ or perithecia were found.

On January 6 plates were poured, using conidia from acervuli which had developed upon an apple inoculated with conidia from the same avocado fruit. A loose white growth of mycelium was produced, bearing very few conidia. These cultures differed conspicuously from plates poured at the same time, using conidia from the avocado fruit. The growth was much more abundant in the latter case and the conidia more numerous, suggesting the possibility that the fungus had lost some of its vitality through its development on the apple. A few setæ were found later and a few apparently immature perithecia finally developed.

On January 6 more plates were poured, using conidia from the same avocado fruit. A luxuriant white growth soon developed, producing an abundance of conidia but no distinct acervuli. Transfers to tubes from these plates produced acervuli with an abundance of setæ and a few apparently immature or sterile perithecia.

On April 30 other plates were poured, using conidia from a leaf taken from a plant in the greenhouse. Germinating conidia were transferred from one of these plates to two tubes. On May 15 both the tubes and the plate showed acervuli with pinkish masses of conidia, and a number of areas of fertile but immature perithecia were found in the plate. The conidia were much more abundant in the tubes than in the plates. Later, a few fertile perithecia developed in the tubes. Transfers of conidia from these tubes to flasks of corn meal grew rapidly and in about a week perithecia formed, but no conidia could be found. A little later the perithecia matured and gave an abundance of ascospores. Plates poured, using these ascospores, produced few conidia and no distinct acervuli, but numerous chlamydospores and an abundance of fertile perithecia.

Many other transfers from this material, both of conidia and ascospores, showed considerable variation in the appearance of the growth and the relative development of conidia and perithecia. Perithecia usually predominated in the plates, whether started from conidia or ascospores. Conidia were more frequently scattered and hyphomycetous than in distinct acervuli. Various attempts were made to determine whether the amount of culture medium used in the plates or the thinness and thickness of the sowing of the spores had any direct relation to the production of ascospores or perithecia. In the case of a strain of the fungus which normally produced perithecia it appeared that perithecia were more numerous and conidia less so in plates which were thickly sown. In about a dozen cases perithecia were found forming along the sides of cotton fibers which happened to be present in the agar. This suggested that the resistance of some solid substance might possibly stimulate their formation. In many cases, especially in this form from Persea, the perithecia showed a tendency to develop about an acervulus, forming a small cluster or group, with the acervulus as a stromatic base.

Two series of generations of pure-line cultures were started from the same original culture of this organism. In one case ascospores only were used and in the other case conidia only. The ascospore cultures were carried through seven generations on the same medium and under practically the same conditions of growth and environment. The conidial cultures were carried through 23 generations and their behavior was compared with the generations from ascospores, as well as with duplicate cultures of each generation. An account of these cultures will be given later under the head of "Pedigreed cultures."

The conidia from leaves in moist chamber varied from 11 to 19.5 by 4.5 to 5.5  $\mu$ . Conidia from cultures varied from 12 to 18 by 4.5 to 6  $\mu$ . Ascospores from leaves averaged about 18 by 6  $\mu$ . Those in cultures ranged from 13.5 to 22.5 by 4.5 to 6  $\mu$ . An ascus and ascospores are shown in Plate I, figures 10 and 10a. Bessey (12)

and Rolfs (66) have referred the conidial form of the fungus on avocado to Colletotrichum gloeosporioides and Bessey (12) reports successful cross-inoculations from citrus fruits and mangos, thus confirming its identity physiologically as well as morphologically with the organism on the orange, which in turn can not be satisfactorily separated from the form on privet, apple, etc. According to the authors cited, infection takes place through the flowers. Higgins (47) has recently described a serious disease of avocado in Hawaii which he considers as probably due to Gloeosporium. Its effect upon the plant, attacking as it does flowers, foliage, and shoots, is the same as that of the organism just described.

## PHASEOLUS VULGARIS L. (WAX BEAN).

Glomerella lindemuthianum Shear. n. comb. Colletotrichum lindemuthianum Sacc. and Magn.

Numerous cultures of the bean anthracnose have been made at different times and in different seasons. The cultures from conidia in most cases have been rather uniform in appearance and behavior, agreeing with the descriptions given by Atkinson (3), Whetzel (95), Edgerton (30), and others. In 16 slant agar tubes made November 3 from different acervuli on 5 bean pods the growth soon became very dark colored. This appears to be quite a constant characteristic of this species, and all the cultures made were practically identical in appearance during their growth. No perithecia were produced in these cultures.

The acervuli varied greatly in number and size in different cultures, and setæ, though usually present, were not numerous. Many chlamydospores were also found in some cultures. Cultures from single conidia, showing the usual appearance of the fungus, are shown in Plate VII.

In December cultures were made in flasks of corn meal by transfer of conidia and a bit of tissue from a bean pod bearing acervuli of the bean-anthracnose fungus. Later, all of the flasks showed good perithecia and asci, but conidia were scarce or wanting. An ascus and ascospores are shown in Plate I, figures 13 and 13a. These cultures finally became contaminated and were discarded. Plates were previously made from them, using the crushed perithecia and asci. The ascospores germinated readily and produced a dense growth of mycelium of the usual appearance. No conidia were found in these plates. Other plates poured from the same ascospore material produced the same typical mycelial growth; and at the end of 12 days perithecia were present in great numbers, but no conidia were found. Other plates were poured on March 16. A single ascospore transferred to a tube and afterwards to a flask of corn meal produced the

usual growth of mycelium; and on April 3 an abundance of perithecia with mature asci were present, but no conidia were seen.

This is the only case in the writers' experience with these organisms in which cultures made from ascospores have apparently produced no conidia or if they were formed they were so few in number that they escaped observation. It does not appear, however, that there can be any doubt about these perithecia belonging to the bean anthracnose. The cultures were originally started from the conidial form on a bean pod and conidia were found in the first cultures with the perithecia. The perithecia, asci, and all the morphological characters of the fungus agree with Glomerella, as will be observed by comparing Plate I, figures 13 and 13a, and also the measurements of conidia and ascospores. The fact that conidia were few in the original culture and wanting in others apparently shows only an extreme variation in this particular, as cultures from different hosts have shown a great degree of variability in respect to the relative abundance of the different spore forms in any race or strain, and there seems to be a general tendency on the part of cultures from ascospores to produce fewer conidia than do those which originate from conidia. In the case of the form from the gooseberry (Ribes oxyacanthoides), described later, the predominance of perithecia was also very striking. Conidia, though present, were usually scattered or formed minute acervuli which were very inconspicuous and easily overlooked, whereas the perithecia were produced in great numbers and were very conspicuous.

In one other series of cultures from conidia from a bean a few small peritheciumlike bodies were found at the edge of the culture, but no asci or ascospores were obtained.

The appearance and behavior of this organism in cultures, as well as the failure of cross-inoculation experiments, apparently shows it to possess sufficiently well-marked characteristics to justify its separation as a distinct species, though the perithecial form taken alone could be distinguished with great difficulty, if at all, from that obtained from other hosts. It is tentatively named Glomerella lindemuthianum Shear.

#### PHORMIUM TENAX FORST.

Glomerella cingulata (Stonem.) S. and v. S. ?Physalospora phormi Schröt.

On April 1 slant agar tubes were inoculated by transfer of spores from an acervulus of Gloeosporium on a leaf of Phormium from the greenhouse. The growth in these tubes had the same general appearance as that of most other forms of this species grown from other hosts. A few acervuli formed with pinkish masses of conidia. Peritheciumlike bodies were numerous in the cultures, but no asci could

be found. Transfers to flasks of corn meal made from one of the above tubes passed through the same course of development and produced peritheciumlike bodies. A few apparently immature asci were found in some of these, but though kept about two months no ascospores were ever seen. Other subcultures from this same series continued to produce conidia and the dark peritheciumlike bodies, but no mature asci were ever discovered. Chlamydospores of the usual kind were present in great abundance in some of the cultures.

Physalospora phormi Schröt. (72) as described from this host agrees with Glomerella and is presumably the perithecial form of the Gloeosporium from which our cultures were made. The conidia

varied from 9 to 15 by 4.5 to 5  $\mu$ .

Gloeosporium phomiforme Sacc. and Ell. also described from this host is apparently a different organism, as the conidia are said to range from 5 to 6 by 3 to 3.5  $\mu$ . There seems little doubt that the species grown by the writers should be referred to Glomerella cingulata (Stonem.) S. and v. S., though no mature asci were seen.

PIMENTA ACRIS (SWARTZ) KOSTEL. (WILD CLOVE).

?Glomerella cingulata (Stonem.) S. and v. S.

On January 19 leaves with small dead areas were taken from a green-house plant, the surface sterilized as usual, and placed in a moist chamber. Acervuli of the usual character of Gloeosporium soon developed on the leaves, and later a few immature perithecia with asci were found, but the spores were not sufficiently developed for positive identification.

Cultures made from the conidia on these leaves produced a growth of the usual character. Conidia were formed, but no accrvuli appeared until the cultures were nearly 3 weeks old. No setæ were found. Peritheciumlike bodies developed in the thickest sown plates, but no ascospores were seen. The conidia ranged from 11 to 17 by 4 to 6  $\mu$ . No fungus of this kind appears to have been reported on this host heretofore.

PIPER MACROPHYLLUM SWARTZ (PEPPERWORT).

 $\slash\hspace{-0.6em}$  Glomerella cingulata (Stonem.) S. and v. S.

On January 30 apparently normal, healthy leaves taken from a greenhouse plant, after sterilization of the surfaces as usual, were placed in a moist chamber. The leaves soon became discolored, and on February 7 numerous acervuli were present. No perithecia were ever found on these leaves.

Slant agar tube cultures were made, using conidia from the leaves in moist chamber. The growth was of the usual appearance of Glomerella cultures, and scattered acervuli with pink masses of conidia appeared. On March 10 about 25 perithecia of different sizes had developed in one tube. These contained immature asci. Subcultures made from this tube showed the same characters as the first, except that numerous setæ were present in the acervuli. These cultures produced many perithecia, but no mature asci could be found. This seems to be the first report of the occurrence of such a fungus on this host.

#### PITCAIRNIA CORALLINA LINDEN.

Glomerella cingulata (Stonem.) S. and v. S.

On January 29 normal, apparently healthy leaves were taken from a greenhouse plant and after the usual treatment placed in a sterile moist chamber. The leaves soon became covered with dark blotches, and a few scattered acervuli developed. No setæ were found. On February 25 fertile perithecia of Glomerella were abundant on both sides of the leaf. No paraphyses were seen. The asci and spores were apparently identical with those from various other hosts. An ascus and ascospores are shown in Plate II, figures 25 and 25a.

Plate and tube cultures made from conidia on the leaves in moist chamber produced a growth of the usual appearance of Glomerella cultures. Acervuli soon appeared. Setæ were abundant in some of these cultures, whereas the original tubes showed no setæ. Chlamydospores also developed and in two corn-meal flasks peritheciumlike bodies occurred, but no asci were found. The conidia from the leaves in moist chamber varied from 12 to 18 by 5 to 6  $\mu$ ; ascospores from the leaves measured 15 to 18 by 4.5 to 6  $\mu$ .

The host indexes mention no organism of this kind on this host.

## PSIDIUM GUAJAVA L. (GUAVA).

Glomerella cingulata (Stonem.) S. and v. S. Glomerella psidii (Del.) Sheldon.

Sheldon (78, 80) has already described the life history of this form. The work of the present writers covers the study of the development of the fungus on leaves in moist chamber and in pure cultures.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On January 29 five apparently healthy leaves were taken from a tree in the greenhouse and after the usual treatment placed in a sterile moist chamber. The leaves soon showed dark blotches and finally became entirely discolored. Acervuli developed first on the petiole and later occurred scattered over the upper and lower surfaces of the leaves. They appeared less numerous and more irregularly distributed than in the form produced on citrus leaves in moist chamber. On April 8 a few perithecia were found on these leaves.

On April 30 two small young leaves in whose axils no flower buds were located, also two leaves a year old in whose axils fruit had been borne, were treated as usual and placed in moist chamber. On May 16 acervuli were abundant on the old leaves, but none were present on the new leaves. Though kept until June 22 no acervuli or perithecia developed on the young leaves, while acervuli and perithecia were numerous on the older leaves. This would suggest the possibility of the fungus having entered the old leaves by way of the flowers of the previous season, while the flowers in the axils of the new leaves not having opened, the new leaves had not yet become infected.

One leaf, in the axil of which fruit had just set, did not develop any Gloeosporium in moist chamber nor did another which bore a flower in its axil.

Four terminal leaves from different shoots situated 1 to 4 inches beyond any fruit and four leaves at the base of fruits, were placed in moist chamber. One leaf in each set developed acervuli. These last experiments do not seem to indicate any clear connection between flowers and fruit and infected leaves in this case. It seems more probable that the dormant infections came from local infection of the leaves.

CULTURES.

Numerous cultures started from conidia from leaves placed in moist chamber at different times during the year, produced a mycelial growth of the usual general appearance of Glomerella. Acervuli were formed in all the cultures, but usually they were not numerous. The spore masses were pink. No setæ were found in any of the cultures. Chlamydospores occurred in some. Perithecia were never found in the cultures.

Cultures made from conidia from fruit of guava in the greenhouse had the same general appearance as those from leaves. Setæ appeared, frequently in great abundance, in nearly all the cultures derived from fruit. Chlamydospores were also abundant in some of these cultures, but no perithecia were found. The conidia from the host ranged from 13.5 to 19.5 by 4.5 to 6  $\mu$ . Conidia from cultures varied from 13.5 to 18 by 4.5 to 6  $\mu$ . Ascospores from the host ranged from 13.5 to 21 by 5.5 to 6  $\mu$ . An ascus and ascospores are shown in Plate I, figures 9 and 9a.

There is nothing, so far as could be determined, in the morphological characters or behavior of this fungus in cultures or on its host to distinguish it from Glomerella cingulata as it occurs on citrus fruits and other evergreen-leaved plants as well as pomaceous fruits. Inoculation experiments reported by Cobb (21) indicated that the fungus could be successfully inoculated into apple, banana, pear, and quince. The form on apple was also successfully transferred to guava by the same investigator.

## RIBES OXYACANTHOIDES (GOOSEBERRY).

Glomerella cingulata (Stonem.) S. and v. S. Gloeosporium ribicolum Ell. and Ev.

On July 5 decaying gooseberries were found at Arlington Farm, Va. Some of these berries showed acervuli of *Gloeosporium ribicolum* Ell. and Ev. and others developed acervuli when placed in a moist chamber.

Test tube cultures were made by transferring conidia from these berries. The growth in the tubes was of the usual appearance of Glomerella cultures. On July 14 acervuli were present in three of the four tubes. They were few in number and located about the point of inoculation. The development of the mycelium was rather more scanty than usual and showed little indication of the dark color which frequently develops in old cultures. These cultures never developed more acervuli, but small groups of perithecia appeared which were either sterile or immature. Transfers were made from each of these tubes to four others. These cultures developed very few acervuli and conidia. Two of these cultures developed distinct stromatic masses of perithecia, all fertile and producing typical ascospores. The massing of the perithecia in these two cultures was very conspicuous, as the perithecia of Glomerella are most frequently either scattered or but slightly aggregated.

Apples were inoculated with ascospores from these cultures, as described later, and the cultures made from the tissues of these decayed apples produced large quantities of perithecia but very few and minute acervuli. The conidia developed were mostly scattered. Plates poured from the cultures from the inoculated apple had a very characteristic appearance. The perithecia developed in great numbers and were quite evenly distributed throughout the medium instead of being aggregated in masses. Acervuli developed on the surface of the medium at certain points, but they were very small and inconspicuous. The perithecia appeared to be rather larger and thicker walled than usual in Glomerella, but the asci and spores as well as conidia agreed in all respects with those from other hosts. The passing of one generation of the fungus through the apple had apparently increased its vitality in some way, as it grew much more luxuriantly in the cultures made from the apple and developed perithecia and conidia much more abundantly.

## RUBUS OCCIDENTALIS L. (BLACK RASPBERRY).

Glomerella cingulata (Stonem.) S. and v. S. Glomerella rubicola (Stonem.) S. and v. S. Gloeosporium rubi E. and E.

Diseased canes of black raspberry received from Shelbyville, Tenn., were placed in a moist chamber. The diseased areas soon produced typical acervuli with dark-colored setæ. Later many perithecia of Glomerella also appeared on the canes.

On March 29 the surface of some of the diseased canes was disinfected as usual, and pieces of the discolored inner bark and wood were transferred to flasks of sterile corn meal. These cultures produced a growth of white mycelium but no acervuli. On April 17 perithecia of Glomerella were present with mature asci and ascospores apparently identical with those found on the canes in the moist chamber.

Other cultures made from conidia on the same canes in the moist chamber produced conidia but no distinct acervuli. Fertile perithecia developed in large numbers over the entire surface of these cultures. Other cultures from the same source and also subcultures continued to produce fertile perithecia in abundance, but conidia were always few and no distinct acervuli were found. Subcultures made from ascospores also produced an abundance of fertile perithecia, but no conidia were found. Chlamydospores also occurred in these cultures. The conidia found ranged from 10.5 to 18 by 5 to 6.5  $\mu$ . Ascospores averaged about 15 by 6  $\mu$ .

The perithecial stage of this form was first produced in culture by Stoneman (89). Several species of Physalospora and of Laestadia have been described from Rubus which do not differ essentially from this Glomerella, so far as the descriptions go. There seems to be no way of separating this fungus from Glomerella cingulata. In the absence of inoculation experiments showing that it will not pass to the apple or other hosts it is referred to that species.

## RUBUS TRIVIALIS MICHX. (WHITE DEWBERRY).

Gloeosporium rufomaculans (Berk.) Thüm. Gloeosporium rubi E. and E.

Early in May, 1909, specimens of white dewberries were received from Macon, Ga. The fruit was soft and had a water-soaked appearance, suggesting a fungous disease. After washing the berries thoroughly in corrosive sublimate, cultures were made on corn meal by transferring portions of the pulp from the berries. One of the flasks to which pulp was transferred, developed a pure growth of Gloeosporium of the usual appearance. The acervuli were numerous and produced pinkish masses of conidia. No perithecia developed in

these cultures. Other specimens of the same diseased dewberries kept in moist chamber also produced acervuli. Poured plates were made with conidia from these acervuli. Acervuli but no setæ were found in the cultures, and no perithecia were produced. The conidia varied in size from 9 to 16 by 4.5 to 6  $\mu$ . In one set of these cultures the conidia averaged rather smaller than in the others, being mostly 9 to 13.5 by 4.5  $\mu$ . This form does not appear to be distinct from that just described from the black raspberry, though the material used in these cultures produced no perithecia. Inoculation experiments described later show that this form adapts itself quickly to the apple.

## SMILAX MEDICA SCHL. AND CHAM.

Gloeosporium rufomaculans (Berk.) Thüm.

On October 31 acervuli of a Gloeosporium were found on leaves of this Smilax in the greenhouse of the Department of Agriculture. Plates which were poured from this material produced a growth of mycelium of the usual character and an abundance of conidia but no distinct acervuli. Transfers were made from these plates to tubes which soon produced large acervuli with pinkish masses of conidia. Later, the mycelium became dark colored, but no perithecia were ever found and no setæ were seen. The conidia ranged from 10.5 to 19.5 by 5 to 6  $\mu$ . The fungus as it appeared on the host and in cultures showed no characters to distinguish it from the conidial forms of Glomerella found on most of the other hosts studied. No species of Gloeosporium or Glomerella seems to have been reported heretofore on Smilax.

## THEA JAPONICA (L.) BAILL. (CAMELLIA).

Glomerella cingulata (Stonem.) S. and v. S. Colletotrichum camelliae Mass.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On January 29 apparently normal, healthy leaves were taken from a greenhouse plant, the surfaces sterilized as usual, and the leaves placed in a sterile moist chamber. They soon began to show a dark discoloration extending from the petiole up the midrib almost to the tip and finally became entirely discolored. Acervuli occurred on all the leaves, and fertile perithecia of Glomerella were also present but not abundant.

#### CULTURES.

Tube cultures were made, using conidia from the acervuli on the leaves in moist chamber, as described above. These cultures produced abundant acervuli but no perithecia. No setæ occurred, though setæ were abundant in the acervuli on the leaf. In other

cultures from the same leaves acervuli and chlamydospores were

found, but no perithecia.

In February leaves of camellia, showing acervuli of Gloeosporium, were received from South Carolina. Cultures were made from the conidia on these leaves. The fungus developed in the usual manner, but produced very few conidia and no distinct acervuli. The mycelium later became dark colored, and finally two perithecial forms were found; one appeared to be Glomerella, while the other produced large brown septate ascospores, indicating that the culture was impure. The Glomerella, however, was apparently identical with that on the leaves and presumably originated from the conidia used. conidia on the host ranged from 13.5 to 18 by 4.5 to 6 \u03bc. Setæ were rarely found. The ascospores from perithecia on different leaves varied greatly. On one leaf they ranged from 10.5 to 18 by 6  $\mu$  and were only slightly curved or inequilateral. On another leaf they were more distinctly curved and ranged from 15 to 28.5 by 4.5 to 9 µ. (See Pl. I, figs. 15 and 15a.) Though the ascospores in this case appear to show a greater range of variation than in some other cases. still there appears to be no good basis for separating this form from that on the orange.

## THEA SINENSIS L. (TEA).

Glomerella cingulata (Stonem.) S. and v. S. Laestadia camelliae (Cke.) Berl. and Vogl. Colletotrichum camelliae Mass.

#### DEVELOPMENT ON LEAVES IN MOIST CHAMBER.

On December 4 leaves with small dead areas were taken from tea plants in the greenhouse and placed in a sterile moist chamber. Acervuli with setæ and perithecia of Glomerella soon appeared on the leaves and many mature asci were found. No paraphyses were seen. An ascus and ascospores are shown in Plate II, figures 16 and 16a.

On April 29 other leaves showing small discolored areas were placed in moist chamber. Acervuli of Glomerella with pink masses of conidia soon appeared on these, and 10 days later an abundance of mature perithecia were found. These perithecia in most cases showed rather distinct but inconspicuous beaks. Paraphyses were also found in these.

Attempts made in February and May to obtain the fungus from apparently healthy tea leaves placed in moist chamber were unsuccessful.

#### CULTURES.

Two cultures on sterile corn meal, made by transfer of conidia from a tea leaf in a moist chamber, produced a few conidia but no distinct

acervuli. Mature perithecia of Glomerella were found in both these cultures in about three weeks. Chlamydospores were also present.

On July 5 a tube culture was started from a single conidium. This developed the usual growth and produced acervuli. Two subcultures were made in flasks of corn meal. These cultures started about as usual and were identical in appearance, but the mycelium began to assume a bluish smoky color rather sooner than in most cases. Acervuli with pinkish masses of conidia also developed. These cultures were kept for nearly a year, but no perithecia were ever found. The conidia on the leaves in moist chamber ranged from 15 to 21 by 4.5 to 6.5  $\mu$ .

Several species of Gloeosporium and Colletotrichum have been described as occurring on tea. They all appear to belong to this Glomerella except Gloeosporium theae-sinensis Miyake which has conidia only 4 to 6 by 2  $\mu$ . The conidial form might be referred either to Gloeosporium or Colletotrichum, as setæ were found on leaves in moist chamber but not in cultures. A microscopic study of the type specimen of Massee's (58) species, Colletotrichum camelliae, leaves no doubt of its identity with the conidial form of the fungus just described.

## THEOBROMA CACAO L. (CHOCOLATE NUT).

Glomerella cingulata (Stonem.) S. and v. S. Colletotrichum theobromicolum Delacr.

On January 19 leaves of cacao or chocolate nut showing small dead areas but no fungus fructifications were taken from greenhouse plants, the surfaces sterilized as usual, and placed in a moist chamber. Many acervuli of Colletotrichum soon appeared, especially along the midrib. Setæ were abundant and also immature perithecia, which later (March 10) showed an abundance of mature asci and ascospores. Paraphyses were also seen. An ascus and ascopores are shown in Plate II, figures 24 and 24a.

Plate cultures were made, using conidia from the leaves in moist chamber just described. Transfers were made from these plates to four tubes in which a growth of whitish mycelium of the usual character developed and soon large acervuli with pinkish masses of conidia appeared at the point of planting in two of the tubes. In the other two no distinct acervuli were found. Setæ were more frequently present on the host than in the cultures. In some of the poured plates no setæ were found. In one tube where setæ were abundant one was found bearing a spore, as sometimes happens in the species occurring on cotton. No perithecia were ever found in any of these pure cultures, and no ascogenous fungus has been described from this host, so far as noted, which seems to agree with the

Glomerella which appeared on the leaves. The conidia from the leaves ranged from 15 to 18 by 6  $\mu$  and agreed in all respects with Colletotrichum theobromicolum Delacr. (26). Two or three other species of Colletotrichum have been described from this host. The conidial measurements given are smaller than those of our organism, and they may perhaps belong to different species. Bancroft (5) has discussed the species on this host.

## VANILLA PLANIFOLIA ANDREWS (VANILLA).

Gloeosporium rufomaculans (Berk.) Thüm.

On January 26 plates were poured, using conidia from acervuli on leaves of vanilla received from Miami, Fla. Setæ were present in some of the acervuli and wanting in others. Acervuli soon developed in the poured plates, and four subcultures were made in tubes. Acervuli soon developed in the tubes, but their number, size, and distribution varied greatly. In one tube there was only one large acervulus, in another the surface of the medium was thickly dotted with acervuli with pinkish spore masses. In some of the tubes the mycelium remained whitish, in others it became quite dark colored. The variations in amount and color of the mycelium, and in the number, size, and distribution of the acervuli were very striking. No perithecia or peritheciumlike bodies were found in any of these cultures. The conidia from the leaves ranged from 13.5 to 24 by 4.5 to 6  $\mu$ .

There are no sufficient morphological characters to distinguish this fungus from that found on the grape and apple. It has therefore been referred to the same species. *Physalospora vanillae* A. Zimm. (97) is apparently the perithecial form.

## VITIS LABRUSCA L. (CONCORD GRAPE).

Gloeosporium rufomaculans (Berk.) Thüm.

On July 30 plates were poured from acervuli of Gloeosporium found on rotten grapes at Washington, D. C. These produced the usual whitish mycelium and numerous acervuli, and a little later perithecia of Glomerella with mature asci and spores were found. Transfers of perithecia from these plates to tubes produced large acervuli and a considerable growth of the dark bluish hyphæ which frequently characterize old cultures. Mature asci and perithecia were found in one of these tubes. In two others perithecia were present but no asci were seen.

On September 25 four plates were poured, using conidia from a single acervulus in the tube just described, which contained the fertile perithecia. Transfers were made from these plates to four other

tubes. Acervuli developed in abundance in tubes a and b. The mycelium was white and rather scanty. In tubes c and d the mycelium was much more abundant and darker colored. Chlamy-dospores were also found in these cultures. On October 16 many perithecia were present in tube a but none in b. The difference between these two tubes was striking, though the cultures came originally from the same acervulus. Variations in the size and shape of the asci are shown in Plate III, figures 30 to 34.

In two cultures out of eight made in August from diseased tissue on small black sunken spots on shoots of a Concord grapevine from Vienna, Va., both acervuli and perithecia of Glomerella were produced. Setæ were found in these acervuli. As other organisms predominated in most of the cultures, it is, of course, uncertain whether any of these cankers were primarily due to Glomerella, which has not heretofore been reported as occurring upon grape shoots, so far as known. Setæ seemed to occur less frequently in the form from the grape and apple than in those from other hosts. Other cultures from grapes from different parts of Virginia produced an abundance of acervuli, and also in one case numerous peritheciumlike or sclerotoid bodies, which always remained sterile. The asci ranged from 55 to 98.5 by 9 to 15  $\mu$ . Asci and ascospores are shown in Plate I, figures 1, 1a, 2, and 2a.

The perithecial form seems to be the *Physalospora baccae* of Cavara (17). The conidial form has been described by the same author as *Gloeosporium physalosporae*.

#### MISCELLANEOUS.

In order to compare the behavior of Glomerella from different hosts in pure cultures, tubes were inoculated February 7 with conidia from leaves of the following six hosts which were growing in the greenhouse: Caryota, Costus, Curculigo, Maranta, Persea, and Piper. These cultures all made a growth practically identical in appearance, and most of the tubes produced acervuli varying more or less in number and size. The mycelial growth was about equal in all. One tube from Costus and one from Curculigo failed to produce acervuli. The difference in these cultures from different hosts was much less marked than is frequently the case in cultures made from a single acervulus on the same host.

On January 29 apparently healthy leaves were taken from nine different hosts in the greenhouse, as follows: Citrus limonum, Citrus decumana, Citrus nobilis, Eriobotrya japonica, Psidium guajava, Thea japonica, Pitcairnia corallina, and Ficus longifolia. The surfaces were thoroughly washed with corrosive sublimate, 1 to 500, and the leaves then placed in sterile moist chambers. On February 15 all the

leaves had developed acervuli of Glomerella and some showed perithecia as well.

From these and numerous other experiments of a similar kind performed at different times during the year with leaves from other plants, it appears that this fungus is quite generally present in the leaves of many plants in a dormant or innocuous condition awaiting some weakening of the host or other favorable condition which may give it an opportunity to develop.

## PEDIGREED CULTURES OF GLOMERELLA CINGULATA FROM AVOCADO.

The characters compared in the pure-line cultures were chiefly the relative abundance and particular characteristics of the conidial and perithecial fructifications. These were all perithecium-producing strains at the start. In some cultures conidia predominated and but few perithecia occurred; in others conidia were few and perithecia predominated. In some the conidia were scattered and hyphomycetous, while in others they were produced in distinct acervuli, frequently large. The perithecia were sometimes separate and scattered evenly and thickly over the surface of the medium; in other instances they were aggregated in dense masses.

The arrangement of the perithecia, whether separate or aggregated in a stroma, has usually been regarded by mycologists as important and has been used as the basis for separating genera and families. All the cultures were grown on the same medium (corn-meal agar) in a culture room under ordinary room temperature and conditions.

# FIRST SERIES OF SEVEN GENERATIONS STARTED FROM A SINGLE ASCOSPORE.

Generations 1 and 2 were very similar in appearance. They produced conidia and an abundance of perithecia evenly scattered over the surface of the medium, as shown in tube 2a, Plate VIII. Generation 1 also showed some submerged perithecia.

The third generation was very similar to the first two but showed more of the deeply submerged perithecia.

In the fourth generation the perithecia, instead of being scattered evenly over the surface, as in the three previous generations, were compacted in black masses, giving the cultures a very different appearance from the earlier generations.

Generations 5, 6, and 7 showed the same characters as generation 4. The variation in arrangement and grouping of the perithecia seems to have been transmitted from generation 4 through these three generations. This series was discontinued at this point. In this case an important variation or mutation suddenly occurred in the fourth generation and was transmitted through three following generations.

#### CONIDIAL GENERATIONS FROM THE SAME HOST.

Generation 1 was started from a single conidium taken from a culture which also produced fertile perithecia. Acervuli and conidia were abundant at first in these cultures and a few perithecia formed in the lower part of the tubes.

Generations 2, 3, and 4 varied but slightly in general appearance and relative abundance of acervuli and perithecia from generation 1.

Generation 5 was practically the same as generation 4.

Generations 6 and 7, consisting of three tubes each, showed acervuli in abundance and lines of submerged perithecia developed in the medium as in some of the ascospore cultures, but they were much fewer.

Generation 8 showed an apparent reversion to the condition of the first five generations, with the same general distribution, arrangement, and relative number of acervuli and perithecia.

Generation 9 was strikingly different from generation 8. Acervuli were few and the perithecia very numerous and thickly scattered over the surface of the medium, resembling most of the ascospore generations. Tube 9a is shown in Plate IX. Two plates poured from this tube showing the same predominance of perithecia are illustrated in Plate XIV.

Generation 10 showed an apparent reversion again to the form in the first five generations. Acervuli were abundant and large; the perithecia were fewer and mostly grouped about the acervuli.

Generation 11, tube b, was practically identical in appearance with generation 10, tube b. Acervuli and perithecia were numerous. Generation 11, tube c, was very strikingly different from generation 10, tube c, showing large acervuli and masses of conidia, but very few perithecia.

Generation 12, consisting of tubes b and c, did not show the characters of the previous generation but rather the reverse, perithecia being abundant and acervuli few.

Generation 13 produced abundant acervuli, but the perithecia were much fewer than in generation 12. The two tubes b and c are shown in Plate VIII.

Tubes b and c of generation 14, derived from generation 13, tubes b and c, respectively, were strikingly different. Tube b produced almost entirely conidia with scattered acervuli, while c consisted almost entirely of perithecia formed along the line of inoculation. These tubes are shown in Plate VIII.

Tubes b and c, generation 15, were very similar to tubes b of generations 13 and 14. A few perithecia were present in each, but they were not numerous and predominating, as in tube c of generation 14.

Of generation 16, tubes b and c scarcely differed from 15. Acervuli and perithecia were present in both. They are shown in Plate IX. Seven subcultures from tube 16 b showed rather regular intergradations from tube 1, which contained perithecia chiefly, to tube 7, which contained chiefly acervuli. These subcultures are shown in Plate X.

Tubes b and c of generation 17 were strikingly and remarkably different. Tube b produced perithecia in abundance, covering the surface of the medium as in most ascospore generations. A very few small acervuli were present. Tube c showed an abundance of large acervuli and a very few scattered perithecia. These two cultures are shown in Plate IX together with their parent cultures 16 b and 16 c.

Of 4 subcultures of conidia, numbered 17 d, e, f, and g, from culture 16 b, three showed perithecia predominating like 17 b, and one had acervuli predominating as in the original 16 b. (See Pl. XII.) Eight more subcultures made from tube 16 b gave four with perithecia like tube 17 b and four of an intermediate character, acervuli and perithecia both being present. Six other subcultures from tube 16 b resembled the parent culture. None of them showed a continuous layer of perithecia as in tube 17 b. Nine subcultures made from conidia from tube 16 c were all very similar in appearance throughout their growth. Acervuli were first produced as in the parent tube, but an abundance of perithecia later appeared. They were aggregated in masses in marked contrast to those in cultures from tube 16 b. Seven of these tubes are shown in Plate XIII. Out of 8 transfers from culture 17 b, which, as mentioned above, produced perithecia covering almost the entire surface of the medium. 7 were almost identical in appearance, showing a great predominance of acervuli and few perithecia, while only one resembled the parent culture. Tube 17 b with 6 of these subcultures is shown in Plate XI.

Tubes b and c of generation 18 grown from tubes 17 b and c, respectively, were very different in appearance. Acervuli predominated in culture b and perithecia in culture c, just the opposite of the parent cultures.

Tubes b and c of generation 19 were about like the ordinary conidial cultures. Acervuli and perithecia were both present, but acervuli predominated. Tube 19 b is shown in Plate XII.

Generation 20 b, from tube 19 b, produced both conidia and perithecia, but the latter were more abundant and covered a broad area on both sides of the culture, as illustrated in Plate XII.

Generations 21, 22, and 23 were all very much like the ordinary conidial cultures in which acervuli predominated and were most conspicuous, although some perithecia were always produced.

These variations might perhaps be regarded as renewed expression of latent hereditary characters or as mutations. If mutations, we

should rather expect them to be transmitted more or less regularly, but they appear for one or a few generations and then disappear without any particular regularity or definite sequence. This behavior would appear to put them in the category of fluctuating variations, which are not supposed to be transmitted. The media and the conditions under which the cultures were grown were made as uniform as practicable in order to eliminate the influence of different factors of environment.

#### OCCURRENCE OF SETÆ AND PERITHECIA IN GLOMERELLA.

Table I.—Record of the writers' observations on the occurrence of setw and perithecia in Glomerella.

Tark	Setæ.		Perit	hecia.		
Host.	Cultures.	Host.	Cultures.	Host.	Miscellaneous.	
Annona cherimola (cheri- moya).	Present					
Brya ebenus (ebony) Caryota rumphiana		Sometimes		Presentdo		
Cinnamomum zeylanicum (cinnamon).						
Citrullus vulgaris (water- melon). Citrus aurantium sinensis	Sometimes	Sometimes		Present		
(sweet orange). Citrus decumana (pomelo). Citrus limonum (lemon)	present.	present.	Present	do		
Citrus nobilis (mandarin)	Few seen, sometimes absent.	Present; not numerous.		do		
Coffee arabica (coffee)		present.				
Costus speciosus (spiral flag). Crataegus sp. (hawthorn)	Sometimes present.	numerous.		do		
Cucumis sativus (cucum- ber). Cucurbita pepo (squash)	Sometimes present.			•••••	No pure cultures.	
Curculigo		present.		Present		
quat). Ficus carica (fig)	Sometimes				Paraphyses pres- ent.	
Ficus elastica (rubber plant).			do		ent.	
Ficus longifolia		present.	Present			
Gleditsia triacanthos (honey locust). Gossypium hirsutum (cot-	Usually	Usnally	do			
ton). Hedyscepe (palm)	present. Sometimes	present.		Present	No cultures.	
Ligustrum vulgare (privet)  Malus sylvestris (apple)	present.	numerous. Sometimes		do	(1)	
Mangifera sp. (mango)	Sometimes present.	present.		immature.		
Maranta arundinacea  Musa paradisiaca sapien-				Present but immature.		
tum (banana). Oxycoccus macrocarpus	Present		Present			
(cranberry). Persea gratissima (avocado)	present.	frmit.	do			
Phaseolus vulgaris (wax bean).	do		do			

<sup>1</sup> A few brown ascospores in culture; chromogenic form found,

 $\begin{array}{c} {\rm Table} \ \ {\rm I.--Record\ of\ the\ writers'\ observations\ on\ the\ occurrence\ of\ set\@innerella.} \\ {\rm Glomerella---Continued.} \end{array}$ 

	Setæ.		Perithecia.		Miscellaneous.
Host.	Cultures.	Host.	Cultures.	Host.	miscenaneous
hormium tenax		on cabbage ted with this	Present; asci immature.		
'imenta acris (wild clove).	form.		Present but	Present but immature.	
Piper macrophylla (pep- perwort). Pitcairnia corallina	Sometimes present.		Present; asci immature.	Present	
osidium guajava (guava)	Present; not numerous.	Abundant on fruit; rare on leaves.		Present on leaves.	
Ribes oxyacanthoides (gooseberry). Rubus occidentalis (black		Sometimes	Present	Present	
raspberry). Rubus trivialis (white dewberry). Smilax medica.		present.			
hea japonica (camellia)		Sometimes present.	Doubtful; culture not pure.	Present	
Thea sinensis (tea)	Sometimes present.	do	Present	Paraphyses present.	(1) (2)
Vanilla planifolia (vanilla). Vitis labrusca (Concord grape).	Sometimes present.	do	Present		

<sup>&</sup>lt;sup>1</sup> Paraphyses present on leaves.

Table II.—Hosts from which ascogenous perithecia of Glomerella as well as conidia have been reported either in cultures or on the host, or both.

<b></b>	Perithecia.			Investigators.	
Host.	Cultures. Host.		Paraphyses.		
Anthurium warocqueanum. Artocarpus incisa. Asclepias syriaca (milkweed). Brya ebeneus (Jamaica ebony). Capsicum annum (pepper)	Present	dododo		Shear and Wood. Stoneman, 1898.	
Caryota rumphiana. Cattleya sp. Cinnamomum zeylanicum (cinnamon). Citrus aurantium (sweet orange).	Present	Present	Present	Maublanc and Lasnier, 1904. Shear and Wood, 1909. Do.	
Citrus decumana (pomelo) Citrus limonum (lemon) Citrus nobilis (mandarin) Coelogyne cristata (orchid) Coffea arabica (coffee)	Present	dodododoPresent on		Edgerton, 1909. Edgerton, 1908.	
DoCostus speciosus (spiral flag). Curculigo spCydonia vulgaris (quince)	Present	Present on		Shear and Wood, 1909. Do. Do. Edgerton, 1909.	
Dracaena Do Eriobotrya japonica (loquat). Ficus carica (fig)	do	leavesdo	Presentdo	Sheldon, 1907. Edgerton, 1908. Shear and Wood, 1909.	
Do	Presentdodo	Present on		Shear and Wood. Shear and Wood, April, 1907.	
Do Do	do	do	• • • • • • • • • • • • • • • • • • • •	771 37	

<sup>&</sup>lt;sup>2</sup> In one case a small spore was found attached to a seta.

Table II.—Hosts from which ascogenous perithecia of Glomerella as well as conidi have been reported either in cultures or on the host, or both—Continued.

Host.	Perithecia.		Dawanhyrana	Tomostinatore	
11056.	Cultures.	Host.	Paraphyses.	Investigators.	
Ficus longifolia		Present		Shear and Wood, 1909.	
Ginkgo biloba. Gleditsia triacanthos (honey	Present			Shear and Wood, 1907.	
locust).					
Gossypium hirsutum (cotton)	do			Do. Edgerton, 1908.	
Do	1	halla		Edgerton, 1909.	
Hedyscepe sp. (palm) Ligustrum vulgare (privet)	Descent	Present		Shear and Wood, 1909.	
	QO	I		Stoneman, 1898. Shear and Wood, 1909.	
Malus sylvestris (apple)	do	On fruit		Clinton, 1902. Von Schrenk and Spaulding.	
		cankers.		1903.	
Do	do	do		Scott, 1906. Shear and Wood, 1907.	
Do				Edgerton, 1908. Shear and Wood.	
Maranta arundinacea.  Maxillaria pieta (orchid)		do		Shear and Wood, 1909. Stoneman, 1898.	
		leaves.		,	
Orchid	Present	Present		Edgerton, 1908. Shear and Wood, 1907.	
(cranberry). Persea gratissima (avocado)	do	Present		Shear and Wood, 1909.	
Phaseolus vulgaris (wax bean).	do			Shear and Wood, 1907.	
Phormium tenax	do	Descent		Shear and Wood, 1909. Shear and Wood.	
Pimenta acris (wild clove)	sterile.				
Piper macrophylla (pepper-wort).	Present			Shear and Wood, 1907.	
Pitcairnia corallina		Present	Present	Shear and Wood, 1909. Sheldon, 1905.	
Do		Present		Shear and Wood, 1909.	
Ribes oxyacanthoides (goose- berry).		1		Shear and Wood.	
Rubus occidentalis (black		Present	1	Shear and Wood, 1909.	
Rubus strigosus (red rasp-	do			Stoneman, 1898.	
berry). Sarracenia purpurea (pitcher		Present on leaves.		Edgerton, 1908.	
plant). Thea japonica (camellia)	Doubtful	Present		Shear and Wood, 1909.	
Thea sinensis (tea)	Present	do	1	Do. Shear and Wood.	
nut). Vanilla sp. (vanilla)		do		Stoneman, 1898.	
Vitis labrusca (Concord grape)	Present			Shear, 1907.	
	<u> </u>	1	1		

#### VARIABILITY OF GLOMERELLA.

The extreme variability of races of Glomerella from the same host which is in some cases greater than that of forms from different hosts renders it exceedingly difficult to make a satisfactory classification of the forms. No character, either morphological or physiological, seems to be well fixed. The appearance of the mycelium in cultures on agar is usually rather uniform. The hyphæ are mostly submerged and white at first, forming a circular colony. After a short time the mycelium frequently becomes dark greenish or smoke colored, and occasionally a pink color develops, as was the case in the chromogenic form obtained from the apple (p. 39). The dark color of the cultures is sometimes due to colored hyphæ and at other times to the presence of the black perithecia or sclerotia and appressoria.

#### CONIDIA.

The conidia show exceeding variability in their manner of production and all their morphological characters. In some cultures they are borne separately on scattered sporophores, suggesting a very diffuse hyphomycete. All intergradations between this form and forms having very large, compact, and distinct acervuli occur. In one strain derived from the gooseberry, certain cultures produced an aerial growth and conidiophores more or less erect and clustered, suggesting in macroscopic appearance a Verticillium. Pure cultures from these conidia, however, produced the usual form with acervuli, which was followed by the development of perithecia and ascospores. In size, shape, and color the conidia are also very variable. They have been found to range from 10 to 42 by 3 to 9  $\mu$  from the same host. They are rarely or never distinctly curved. When scattered on a slide they usually appear colorless, but vary from a very pale-cream to a brightsalmon color in masses. Occasionally a few dark-colored conidia have been found

#### SETÆ.

Setæ are frequently entirely wanting in some cultures while abundant in others from the same host. Some acervuli from one single-spore culture may show many setæ, others few, and still others none. The setæ also vary greatly in size, length, and septation. In two forms, those from cotton and Theobroma, they have been found bearing conidia.

### APPRESSORIA, OR CHLAMYDOSPORES.

The appressoria, or chlamydospores, are also exceedingly variable in size and shape. As has been pointed out by Hasselbring (45) and others, their occurrence appears to bear some relation to lack of available nutriment and to contact with some hard surface.

In water drop cultures on glass slides they usually develop either from conidia or ascospores in 24 to 48 hours. (See figs. 2 and 3.)

A germinating chlamydospore is shown in figure 4.

#### YEAST AND OTHER FORMS.

Viala and Pacottet (92) have reported the occurrence of yeast forms of Gloeosporium. Yeast forms have never occurred in any of the writers' pure cultures. They have occasionally been found, but only in cultures made directly from fruit, in which cases they were evidently contaminations. The same authors also report the production of spermagonia and pycnidia in *Colletotrichum lindemuthianum*. No fructifications of this kind have ever been found by the writers in pure cultures of Glomerella. The writers have never seen the endospores described by Sheldon (79) and confirmed by Taubenhaus (91).

#### PERITHECIA.

Perithecia have been found to vary in abundance, size, shape, arrangement, and location with reference to the surface of the me-

dium. They are generally globose or subglobose, though sometimes elongated or pear shaped. The beak when present is usually very short, though commonly the perithecia are merely papillate. In rare cases, however, they have been found with distinct elongated beaks similar to those illustrated by Stoneman (89). On the host plant they have always been found embedded

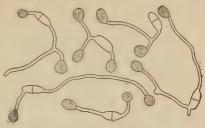


Fig. 2.—Germinating conidia of Glomerella cingulata from a culture from pear, showing the formation of chlamydospores after 24 hours in drop cultures of sterile water.

in the tissues. In artificial cultures they are usually embedded but occasionally form erumpent, superficial masses. Sometimes they

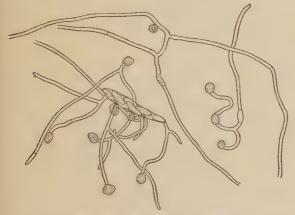


Fig. 3.—Germinating ascospores of *Glomerella cingulata* from cultures from gooseberry, showing formation of chlamydospores after 55 hours in drop cultures of sterile water. Ascospores usually produce longer germ tubes than conidia.

are evenly scattered and entirely separate, as shown in Plate XV; at other times they are aggregated and seated upon a more or less distinct stromatic base. Occasionally they are almost sterile or are represented by simple black, solid, sclerotoid bodies.

ASCI.

The asci are also

extremely variable in size and shape, as will be observed by a comparison of Plates I, II, and III and the measurements of speci-

mens from the same and different hosts, as recorded in Table III. The apex of the ascus shows a peculiar structure which apparently has some relation to the expulsion of the spores. This feature



Fig. 4.—A germinating chlamydospore, or appressorium, of Glomerella cingulata from a culture from gooseberry, showing the development of the germ tube after 24 hours in cornmeal agar.

the spores. This feature has been discussed by Koorders (54).

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#### ASCOSPORES.

The ascospores show almost as great variation in size, shape, and other characteristics as do the conidia. They range from 9 to 24 by 3 to 7.5  $\mu$ . The contents are sometimes rather coarse and granular, at other times more homogeneous. Vacuoles or oil drops are frequently present, sometimes one large one in the center or at each end, at other times two smaller ones at each end. Ascospores are almost always slightly curved. They are usually almost colorless, but when old and in mass they frequently show a pale-lemon or salmon color, and occasionally very old spores are decidedly dark colored.

#### PARAPHYSES.

The early investigators of Glomerella do not mention the presence of paraphyses. It will be seen by consulting Table II that these bodies have not been very frequently found in our material or reported by others. When observed they appear to be very thin walled and are not always easily discerned. Their presence or absence does not seem to be sufficiently constant to be of much taxonomic value.

#### HOST RELATIONS.

Most of the forms from various hosts appear to be able to infect other hosts under certain conditions, at least, as indicated in Table IV, which gives the results of cross-inoculation experiments of other authors as well as of the writers. With a few exceptions, such as the bean and cotton anthracnose and perhaps the form from the squash, different races from the same host seem to vary as greatly in virility as do forms from different hosts.

#### CAUSES OF VARIATIONS.

In dealing with the phenomena of heredity and variation in Glomerella there is apparently no reason to believe that the Mendelian theory is involved or that heterozygosis takes place, as no union of nuclei between different individuals or species is known to occur. Edgerton (32) has recently expressed his belief in a cross-fertilization between two strains of Glomerella from Populus, but the evidence given is not conclusive.

It seems clear that whatever fusions may occur between nuclei in the development of the individual organism which arises from a single spore, such fusions, though they be admitted to represent sexual union, could scarcely be conceived to add or transmit any new characters to the resulting progeny unless they were characters acquired by one of the fusing nuclei and not by the other during the life history of the individual. It is difficult to conceive how the union of two closely related nuclei originating in the same individual or the same cell could add to the essential characters of the organism in any way.

Individuals originating from single spores of Glomerella must be regarded as homozygous so far as it is possible to ascertain at present, i. e., no union of nuclei or gametes between different individuals, races, or strains has been proved.

Whatever differences the progeny of such individuals might show would evidently be due to mutation or some other internal cause, such as the renewed expression of latent hereditary characters, or else to some external or environmental action.

As it has been impossible to trace any causal relation or connection between most of the phenomena of variation observed and the conditions of environment to which the organisms were subjected, it must be concluded that the inducing causes are internal or else so obscure as to escape observation. In any case the evidence accumulated by others as well as by the writers appears sufficient to justify the conclusion that many of the variations observed and reported here are not entirely due to any effect of simple environmental factors. It must be remembered, however, that it is difficult if not impossible at present to standardize media and methods with sufficient accuracy to positively determine the exact effect of environmental factors on these fungi. Gorham (38) and others have pointed out the apparent impossibility of standardizing media containing agar and other organic compounds. Synthetic media of pure inorganic chemicals can not be substituted for many fungi, as they do not usually fruit on such media. More work is needed to verify the conclusions of Stevens and Hall (87) and others in regard to the direct effect of modifications of the chemical constituents of culture media on the behavior of parasitic fungi. The problem is evidently far more complex than some investigators have appreciated, and its complete solution can scarcely be hoped for in the near future, but the most hopeful line of attack seems to be that of pedigreed cultures of asexual or unisexual organisms grown and observed under the most exactly determined and controlled conditions possible and in sufficiently large numbers and through enough generations to reduce probable errors from accidental causes to a minimum.

The work of Jennings (49) with Paramecium and that of Barber (6), Will (96), Beijerinck (11), and Hansen (44) on yeasts, as well as that of other authors cited by Pringsheim (65), demonstrate at least one thing, and that is the actual existence of rather distinct races or strains within species. These races possess more or less distinctive and constant morphological or physiological characteristics which are generally inherited by their progeny and are apparently not primarily dependent upon environmental conditions.

Table III.—Measurements of conidia and ascospores from cultures and from the hosts, showing the ordinary range of variation.

one wing the or wind g , unique of						
	Con	idia.	Ascospores.			
Host.	Cultures.	Host.	Cultures.	Host.		
Annona cherimola (cherimoya).	12-16.5 by 4-4.5	12-19.5 by 4-4.5	μ	μ		
Brya ebenus Caryota rumphiana Cinnamomum zeylanicum (cin- namon).	10.5–12 by 3–4	12-19.5 by 4.5-5 13.5-25 by 4.5-6 12-21 by 5-6		12–18 by 4.5–6. 16.5–21 by 6.		
Citrullus vulgaris (watermelon). Citrus aurantium sinensis (sweet orange).	9–12 by 4.5–5 10.5–18 by 3–4.5			12-18 by 4.5-6.		
Citrus decumana (pomelo) Citrus limonum (lemon) Citrus nobilis (mandarin)	14-17 by 4.5-7.5 10.5-18 by 4-5 11-19.5 by 4.5-6	13-18 by 4.5-6		18–23 by 4.5–6. 13.5–19.5 by 4.5–6. 16–19.5 by 4.5–6.		
Coffea arabica (coffee) Costus speciosus (spiral flag) Cucurbita pepo (squash)	15–18.5 by 4.5–6. 10–16.5 by 4.5 8–30 by 3–4.5	12-18 by 4.5-6		15–18 by 5–6. 15–21.5 by 5–6. 12–24 by 5.5–6.		
Curculigo sp Eriobotrya japonica (loquat) Ficus carica (fig)	10.5–16.5 by 3.5–	6.75, 13–19 by 5–6 12–15 by 4.5–5	13.5–19.5 by 4.5–6	12-19 by 4.5.		
Ficus elastica (rubber plant) Ficus longifolia	4.5. 10.5–22 by 4–6 12–15 by 4–6	12-15 by 4.5-6 16-20 by 6	13.5-24 by 5-6	11–18 by 4–4.5. 12–20 by 3–4.		
Ginkgo bíloba	10–22.5 by 4.5–6.	11-21 by 5-7.5 13.5-16.5 by 4.5-5	11-21 by 4.5-6.5 12-24 by 5-7			
Gossypium hirsutum (cotton) Hedyscepe sp. (palm) Ligustrum vulgare (privet)	i0-42 by 3-9 12-36 by 6-7	12-25.5 by 4.5-6 13-14 by 4-5 12-21 by 4.5-6	15–18 by 4.5–6 13.5–23 by 4.5–5.5	12–19.5 by 4.5. 13–19.5 by 5–7.5. 13.5–18 by 4.5.		
Malus sylvestris (apple).  Mangifera sp. (mango).  Maranta arundinacea.  Musa paradisiaca sapientum	12-18 by 4-4.5 12-25.5 by 3.5-6 9-28 by 3-6.5	9–18 by 3–6 12–25.5 by 3.5–6. 5–7.5 by 2–3 12–19.5 by 4.5–7.5	12–19 by 4.5–6	13.5-19 by 4.5-7.5.		
(banana). Oxycoccus macrocarpus (cranberry).	12-18 by 4.5-6	10.5-21 by 4.5-6	9-20 by 5-7.5			
Persea gratissima (avocado) Phaseolus vulgaris (wax bean) Phormium tenax	12-18 by 4-6 10.5-16.5 by 4.5-5 13.5-18 by 4-4.5	12-18 by 4-5 12-16.5 by 4.5-6 13-19.5 by 4-5.5	10.5–16.5 by 3–4.5 15–22.5 by 4.5–6.	13.5–18 by 4.5–7.5.		
Pimenta acris (wild clove) Piper macrophyllum (pepperwort).	11-17 by 4-6 10.5-15 by 4-4.5	10.5-15 by 4-5				
Pitcairnia corallina. Psidium guajava (guava). Ribes oxyacanthoides (gooseberry).	12-22.5 by 3-4.5 13.5-18 by 4.5-5	13.5–18 by 4.5–5 12–19.5 by 4.5–5	13.5–19.5 by 4–5.	16.5–18 by 4.5. 13.5–19.5 by 4.5–5.		
Rubus occidentalis (black raspberry). Rubus trivialis (white dewberry).	9–16 by 4.5–6	-	13.5–19.5 by 4.5–5	15-18 by 4.5-5.		
berry). Smilax medica Thea japonica (camellia)	15–25.5 by 3–4	12-22.5 by 3.5-6 12-19.5 by 4-5 12-16 by 4.5-5	12-19.5 by 4.5-6	12-16.5 by 4-6.		
Thea sinensis (tea) Theobroma cacao (chocolate nut).	12–15 by 3.5–5 10.5–13.5 by 4–4.5	15-18 by 4.5-6 13.5-18 by 4.5-6		10.5-24 by 4-7.5. 15-24 by 4.5-7.5.		
Vanilla planifolia (vanilla) Vitis labrusca (Concord grape).	10.5–20 by 4.5–6	12-24 by 4-6 12-15 by 4.5	12-21 by 4.5-8			

Note.—The maximum and minimum measurements given could undoubtedly have been increased in many instances, as no special effort was made to determine the extremes in most cases.

### THE PRODUCTION OF PERITHECIA IN GLOMERELLA.

The production of perithecia in Glomerella is a matter of considerable interest and importance, apparently involving some of the fundamental problems of evolution and development. Why is it that the full life cycle of this and various other pyrenomycetes is sometimes completed in pure cultures on sterile media, while at other times only conidia or pycnospores or no fructifications of any kind

are formed? Various explanations and suggestions have been made by different writers at different times, but in most cases there has been no sufficient experimental evidence offered to establish fully any of the theories advanced. A brief review of the various attempts which have been made, and the suggestions offered, may be of some interest in this connection. Klebs (53), Smith (83), Brefeld (14), Stoneman (89), Appel and Wollenweber (1), Butler (16), Glück (37), Ihssen (48), and others have discussed the subject.

The production of the sexual fructifications of certain algae and aquatic fungi may perhaps depend primarily upon factors of nutrition or environment which can be controlled under cultural conditions. The work of Klebs (53), especially, seems to justify such a conclusion. Much more work is necessary, however, to thoroughly verify this and especially to eliminate the possibility of some of the results being due to the use of different races or strains. Kauffmann (50) in the account of his studies on Saprolegnia, expressed the belief that their behavior depended entirely upon definite chemical and physical conditions readily controlled. He also thinks that culture media and conditions could be standardized and safely used as a basis for species segregation. This apparently needs further verification, as Lechmere (57) was unable to obtain the same results with these organisms.

In the case of the sexual forms of alge some writers have attempted to show that there is a more or less regular periodicity in the appearance of the fertile fructifications of different species. Recent investigations by Danforth (22) appear to throw doubt upon the general application of this view also. However the case may be with the alge, there is little or no evidence at present available to show that periodicity is an important factor in the production of the perithecial forms of Glomerella and other pyrenomycetes. In the writers' culture work with Glomerella and other genera, such as Guignardia, Melanops, and Cryptosporella, when mature spores are used, they have been unable to find any relation between the time of the collection or the age of the material and the production of the perithecial forms. Under natural conditions it is known that perithecia are in general most abundantly produced in temperate regions during late winter and spring.

Brefeld (14) suggested as the result of observations of ascogenous fructifications under natural conditions that their production depended to some extent on the season of the year and the substratum; but in our experience, cultures made from fresh material collected under natural conditions do not produce perithecia any more frequently from material collected at one time than at another. Various experiments in growing different strains of Glomerella and other pyrenomycetes on various substrata do not indicate that this

factor either determines the production of perithecia, though a fruiting strain will fruit more abundantly and freely on some substrata than on others.

It has also been suggested that the nearness or remoteness of origin from ascospores of conidia used in any particular series of cultures might be a factor of importance in determining the course of development of such cultures. The data bearing upon this question which we have obtained from our pedigreed pure-line cultures carried through several generations do not appear to support this theory, though perhaps the available data are not sufficiently numerous to

justify definite conclusions.

Of a similar nature is the idea that the production of ascogenous forms occurs only at intervals after the virility or vitality of the conidial or pycnidial form has been greatly reduced by continuous asexual reproduction. There is no known evidence to support this view. Whether there is an actual or potential sexual stimulus or union of nuclei involved in the production of perithecia in Glomerella is not known. No work has been done on the cytological features of the development of the perithecia. As perfect fertile perithecia are produced in abundance in pure cultures from single spores, either conidia or ascospores, it seems certain that no fertilization or union of nuclei between different individuals is necessary. Whatever nuclear unions take place must be between nuclei of the same individual, as is known to be the case in some other ascomycetes.

The most general supposition has perhaps been that which predicated the production of perithecia as dependent upon certain conditions of nutriment or other environment of the organism. This has led to the trial, by most investigators, of various culture media of different constituents in different proportions and also the submission of cultures to various conditions of temperature, air, light, and moisture. Very little success with pyrenomycetous fungi has ever been attained in this direction. In no case have the writers been able to cause perithecia to appear in cultures which did not produce them when grown on corn-meal agar under ordinary laboratory conditions. So far as we have been able to determine, no cases have been reported in which the evidence was sufficiently conclusive to prove that perithecia were produced in artificial cultures as the direct result of modifications of the culture media. Smith (83) reports that the formation of perithecia in a fertile race of Neocosmospora was prevented by the use of strongly alkaline media, but no nonperithecia-forming strains were made to produce perithecia by change of medium.

Appel and Wollenweber (1), in discussing this problem as it relates to Fusarium, Nectria, Neocosmospora, etc., have made some very ingenious suggestions in attempting to account for the production or nonproduction of perithecial forms in cultures. One is that the forms which produce perithecia in cultures on artificial culture media are pure saprophytes, whereas the forms which do not produce perithecia in cultures on artificial culture media are parasites which require the living host or living plant tissues to complete their development. This scarcely accords with the well-established fact that there are all grades and degrees of parasitism among fungi, from the highly specialized obligate forms, such as rusts and smuts, to the weak facultative forms which are only parasitic under certain conditions and in certain stages of their development and may be saprophytic under other conditions or in other stages. A large part of the pyrenomycetes, though parasites, are not of a high type. They frequently pass the early stages of their development—that is, their conidial and pycnidial conditions—as parasites on the living host, whereas they only complete their life history and produce their perithecial or sexual forms upon the dead tissues of the host after it has been killed either by the direct action of the fungus or by some other cause. This fact is emphasized by Massee (59) in his discussion of the evolution of parasitism in fungi. It would not, therefore, appear remarkable if many of these forms should complete their life cycles on sterile culture media containing the essential nutrient substances required by the organism. There is apparently no reason to suppose that the case of Fusarium and Nectria is not directly comparable in this respect with that of Gloeosporium and Glomerella.

There seems to be an abundance of proof to establish the parasitic character of many forms of Gloeosporium. In case of the bitter-rot of the apple, for instance, this parasite has been obtained directly from the tissue of growing fruits before their maturity and when transplanted on artificial culture media has produced perithecia as well as conidia. Perithecia are also frequently produced upon fruits which have been destroyed by the parasite. The suggestion, therefore, that in cases where a Fusarium does not produce perithecia in culture media it is a parasite and that when a Fusarium does produce perithecia in culture media it is a saprophyte, does not appear to accord with what is already known in regard to Glomerella and some other pyrenomycetes.

The case of Neocosmospora, as described by Butler (16), is scarcely more conclusive. The fact that he did not succeed in infecting plants by the use of ascospores of Neocosmospora in the few attempts made does not necessarily prove that this fungus is not a parasite and that it is specifically distinct from the Fusarium form with which he made successful inoculations. As is well known to plant and animal pathologists, there is a great range of variability in the virility or capacity for infection of various races, strains, or forms of what are regarded as the same species; at least they must be so regarded

unless some other basis than morphological differences and differences in behavior in culture media can be established as a basis for species segregation. It should be shown either that there are morphological differences between the conidial form of the Fusarium causing the wilt and the conidial form of Neocosmospora sufficient to identify and separate them, or else a large series of inoculation experiments should be carried out, using ascospores and conidia from a variety of sources and including a sufficient number of strains to justify the conclusion that the fungus will not infect the host and may be considered a physiological species.

The other suggestion of the same authors is that the species of Fusarium with which they were working and which did not produce perithecia in cultures may be autonomous forms which have no perithecial stages and that their close resemblance to the conidial forms of Nectria, Neocosmospora, and related genera may be a mere superficial one which does not necessarily imply any direct relationship. This is ingenious also, but unfortunately it is incapable of experimental proof and is not in harmony with the present trend of myco-

logical facts and opinions.

The connection between various conidial and pycnidial forms and their ascogenous stages is being slowly but surely proven by pure-culture methods, and it seems much more natural and reasonable to suppose that the majority at least of the so-called "imperfect fungi" are stages in the life history of ascomycetes and hymenomycetes, though comparatively few have yet been positively connected and the determining factors concerned in the complete development of the organisms are still unknown.

The production of perithecia in the numerous cases of Glomerella which are reported here is evidently a fairly well-fixed hereditary racial character. Having obtained by repeated cultures from different collections a race or strain which produces both conidia and perithecia on culture media, other generations grown from either the conidia or ascospores of this strain continue to produce both spore forms of the fungus indefinitely. In one case, Glomerella cingulata from Persea, as already described, a race was grown through 23 successive generations and still produced both conidia and ascospores in as great abundance as at first.

Miss Wakefield (93), as a result of her investigations of Schizo-phyllum commune and Stereum purpureum, finds also certain races which show a much greater tendency to produce spores than others under the same conditions of culture. Brefeld (14) states that Penicillium produces ascogenous fructifications at one time while at another, under exactly the same conditions, it produces only conidia.

All the writers' work with Glomerella, as well as with other pyrenomycetes, indicates that the production of perithecia is a hereditary

racial character which does not depend primarily on special conditions of nutriment or environment. This conclusion does not. unfortunately, bring us much nearer to the real cause of the phenomenon; it merely eliminates some of the factors heretofore regarded as controlling. The real nature of the inducing causes (for such must be supposed to exist) which determine whether a race shall produce ascogenous or only conidial fructifications is still unknown. Most frequently a strain producing fertile perithecia produces them in abundance. All sorts of intermediate conditions, however, occur from strains which produce only sterile peritheciumlike bodies or sclerotia to those which produce quite constantly great quantities of fertile perithecia. It is, of course, not certain that their behavior in nature corresponds in this respect to that under artificial conditions. Not all of the cultures started with conidia taken from acervuli which were associated with fertile perithecia on the same leaf produced perithecia. There is no certainty, however, that the acervuli and perithecia arose from the same strain, as the manner of the development of the fungus on leaves in moist chamber appears in many cases to indicate that there are more or less numerous points of dormant infection from which the fungus develops and the growth from these finally covers the whole leaf, as shown in Plate V. The intermingling of fructifications originating from different infections perhaps explains why no perithecia were produced in a number of cases in which cultures were made from leaves showing both acervuli and perithecia more or less mixed. No ascogenous strain has ever appeared in any of the hundreds of cultures of conidial strains which have been grown under various conditions on different substrata. Not having had an opportunity to observe the transition from a nonascogenous to an ascogenous race, any attempt to account for this change, which apparently must occur, would be purely hypothetical. The existence of certain intermediate conditions might suggest the gradual development of these ascogenous races, while the failure to produce any evidence that cultural or other environmental influences determine their production seems to indicate a deeper and more obscure cause for their origin. Ascogenous strains may perhaps arise as mutations. This suggestion, however, throws no light upon the cause of their origin.

It is possible that a knowledge of the nuclear phenomena occurring during the stages of development preceding ascus formation may throw some light upon this problem. The possibility of the existence of plus and minus strains uniting as in Mucor has been considered. In many cases where colonies originating from separate ascospores or conidia of perithecial strains meet in a plate culture there is a greater development of perithecia along the line of contact than in

other parts of the colonies (Pl. XV); but in no case have perithecia been produced under such conditions when the conidia belonged to a race of the fungus which did not produce perithecia in the separate colonies. Edgerton's (32) experiments along this line already referred to are not conclusive. That no such union of different strains is necessary or essential for the production of perithecia seems certain from the fact that perithecia develop in abundance in a colony derived from a single conidium or ascospore of a perithecial race. Until it has been demonstrated that the increase of perithecia at the point of contact between two colonies is due to nuclear fusions between the two growths it seems preferable to attribute the phenomenon to some simpler cause such as the greater exhaustion of the nutriment at the point or some other slight stimulus. For the present we must admit our utter ignorance of the determining factor or factors concerned in the production of ascogenous fructifications in Glomerella. Certain previously supposed factors, however, such as have already been discussed here, have in most cases been sufficiently tested to justify their elimination. This helps to simplify the problem somewhat and suggests research in other directions which may possibly prove more profitable.

#### INOCULATION EXPERIMENTS.

Inoculation experiments with Glomerella, either in the field or greenhouse, are not always conclusive. Under proper conditions and with plants known to be free from dormant infections, inoculations, by surface application of spores, under optimum conditions of temperature and moisture, should give the most trustworthy results. To be certain, however, that a plant is free from dormant infection, it must be grown from seed under conditions which would preclude the possibility of infection from any source except the inoculation.

Most of the inoculation experiments of the writers have, however, been made with fruits or plants growing under ordinary laboratory or greenhouse conditions. As it is a practical impossibility to determine whether any particular plant or part of a plant is entirely free from infection, under such conditions, the results obtained can not always be regarded as conclusive. In most of the experiments described here, inoculations were made by insertion of conidia or ascospores of Glomerella in the tissue of the host. Such experiments with immature fruits when made under proper conditions and with sufficient checks are believed to give evidence of some value in determining the host relationships of the organisms. In the case of perfectly mature fruits the significance may not be so great.

Since it has been found that different races or strains of Glomerella vary exceedingly in virility and also that different host plants and varieties of the same host show great differences in susceptibility to any particular race of the fungus, it is clear that success or failure of inoculation experiments may depend, in many cases, on some of these factors. Further discussion of this subject will be found under the heading "Parasitism of Glomerella." Proper conditions of temperature and moisture are also necessary for successful infection either by surface application or puncture. Negative results from inoculation experiments with spores applied to the uninjured surface of a host can not be regarded in all cases as sufficient evidence that the plant is not susceptible to the disease, as the infection may really occur but not develop further at the time for lack of proper temperature, moisture, or host conditions.

In the inoculation experiments in which fruit was used, it was, in most cases, nearly or quite mature. The specimens were always thoroughly washed with corrosive sublimate, 1 to 500, to destroy any spores which might be present on the surface. They were then rinsed with distilled, or sterile, water, and placed in a sterile chamber. A small beaker of water was also placed in the chamber to afford a slight amount of moisture. Unless otherwise stated, the fruit inoculations were always made on picked fruit with a sterilized needle, conidia or ascospores being inserted in a small puncture through the skin. Checks were used which remained free from rot in all cases except where some special statement is made to the contrary.

#### APPLE TO APPLE.

Various inoculations of apples were made at different times by the writers, conidia of Glomerella from different races or strains being used, in order to compare the rapidity of their development and the effect upon the host. In all the cases in which the inoculations were made by punctures, infection occurred and decay followed. The fruit had the usual appearance of bitter-rot, and acervuli were usually produced. The spots developed to about 1 centimeter in diameter in a week. In some cases development was slower than when inoculations were made with conidia from other host plants.

In three series of experiments conidia were applied to the unbroken skin of nearly mature apples. In none of these cases did any rot follow, although the fruit was kept in a moist chamber for a long period.

Sound apples were inoculated by puncture, using conidia from a single spore culture of the chromogenic form of the apple Glomerella, which has already been described on page 39. Rot developed about each puncture with about the same rapidity as in other cases and acervuli were produced in about three weeks.

Clinton (19) reports successful inoculations of green fruit on trees by inserting conidia in punctures, as indicated in Table IV. He also reports in the same place successful inoculations with ascospores inserted in punctures, and he succeeded in infecting green fruit picked from the trees by placing conidia in drops of water on the unpunctured skin. In two experiments, however, no infection followed spraying the spores on the surface of green fruit. In one experiment he reports successful infection by applying ascospores to the unbroken surface in a drop of water.

Von Schrenk and Spaulding (70) report the successful inoculation of apple limbs by the introduction of ascospores in slits in the bark. They also report successful infections on healthy fruit with spores inserted in punctures and one successful experiment in infecting picked fruit in a moist chamber by spores applied to the unbroken skin. Scott (73) also has made successful inoculations of apples with conidia

without puncture.

It will be noted that very few cases have been reported of successful infection of apples without punctures, and much more information is needed in regard to the exact time and method by which infection of the apple takes place. The occurrence of conidia-producing cankers on branches is too rare in most cases, in the East at least, to account for the great prevalence and immense number of the infections which are found during favorable seasons.

#### APPLE TO BEAN.

Halsted (40) reports successful inoculation of bean pods from apple by the introduction of a portion of the decayed tissue or spores. This is the only successful inoculation of beans with the apple anthracnose that has been reported, and in view of the failure of other investigators to secure the same results it does not seem advisable without verification to give it much weight in determining the specific relationship between the two forms occurring on these hosts.

#### APPLE TO GRAPE.

On October 14 eight berries of mature Niagara grapes were inoculated by puncture with conidia from apples. Eight others were inoculated by puncture with conidia from a culture. At the end of two weeks the first eight all showed rot and acervuli had formed on five berries. At the end of the same period the other lot all showed rot, but only two bore acervuli.

#### APPLE TO PUMPKIN.

One mature pumpkin was inoculated by puncture with conidia of Glomerella from an apple. No definite result was obtained, as the pumpkin soon decayed from other causes.

#### AVOCADO TO APPLE.

On December 17 three apples were inoculated by puncture, using conidia from a fruit of avocado. At the end of the week all showed decayed spots 1 inch in diameter at the point of inoculation with acervuli present in two cases. The other spot was smaller with no acervuli present at first, but many developed a little later.

# AVOCADO TO BEAN.

On June 4 six young pods of wax bean were inoculated with conidia. No signs of infection ever appeared.

#### AVOCADO TO CABBAGE.

On November 8 young cabbage plants, bearing 8 to 10 leaves, were inoculated with conidia from a pure culture. Inoculations were made by puncture and by surface application. No signs of infection ever appeared on the inoculated leaves.

#### AVOCADO TO COTTON.

On April 1 three open cotton flowers were inoculated by applying conidia in a drop of sterile water to the stigma of the flower. The flowers were then covered with paper bags. No signs of infection had appeared at the end of two weeks, and no conidia developed on the bolls when removed and placed in moist chamber.

On November 8 bolls one-half grown were inoculated with conidia from a pure culture. Part of the inoculations were made on the surface, the others by puncture. No signs of infection appeared at the end of a month.

#### AVOCADO TO RUBBER PLANT.

Leaves were inoculated with conidia from pure culture, some applied to the surface and some by punctures. No signs of infection followed in either case.

#### AVOCADO TO TEA.

On November 8 young leaves were inoculated by both surface and puncture methods with conidia from a pure culture. No signs of infection appeared at the end of a month.

#### BEAN TO APPLE.

On September 30 four apples were inoculated by puncture with conidia from a bean pod. No signs of infection had appeared at the end of a month.

On July 25 seven apples were inoculated by puncture with conidia from a green bean pod. These fruits decayed after a month but no accervuli formed. Cultures made from the decayed spots produced only a sterile mycelium somewhat resembling that of Gloeosporium lindemuthianum. Positive identification was impossible without spore formation.

On October 4 twelve Willow Twig apples were inoculated by puncture with conidia from a bean pod. No signs of infection followed.

On October 6 four Willow Twig apples were inoculated on the surface with conidia from a bean pod. At the end of a month no signs of infection had appeared. The same experiment was repeated November 2 with the same result.

On October 6 four apples were inoculated by punctures with conidia from a bean pod. No signs of infection had appeared at the end of a month.

In December three Willow Twig apples were inoculated by puncture with conidia from a bean pod. No signs of decay occurred at the end of a month.

Later, three other mature apples were inoculated by puncture with conidia from culture. No decay followed and no acervuli developed. Twenty-nine other apples were inoculated by puncture with conidia of the bean Glomerella at different times during the season. In no case did decay or development of acervuli follow. In eight other cases also where conidia were applied to the surface of apples no infection occurred. These experiments seem to indicate that the Glomerella on the bean is physiologically different from that on the apple.

#### BEAN TO BEAN.

On June 4 six young pods of wax beans 2 inches long were inoculated by applying conidia from a bean pod to the surface. The plants were covered with a bell jar. No signs of infection ever appeared on these pods.

On June 5 the same experiment was repeated with the same result. The reason for the failure of these inoculations is not clear.

# BEAN TO COTTON.

On July 16 four young bolls were inoculated by puncture with conidia from a bean pod. Small, dark, sunken areas developed about the point of inoculation in all, the same as with inoculations from cotton to cotton made at the same time. No acervuli appeared except on one of the bolls after removal to a moist chamber. These acervuli may not have arisen from the original inoculation from the bean.

#### BEAN TO PUMPKIN.

A mature pumpkin was inoculated by puncture with conidia from a culture from bean. No signs of infection followed.

# BEAN TO HUBBARD SQUASH.

A mature squash was inoculated by puncture with conidia from a culture of the bean Glomerella. No decay followed and no acervuli formed.

# BEAN TO TOMATO.

Four green tomato fruits were inoculated by puncture with conidia from wax beans. No decay followed and no accervali formed.

# BEAN TO WATERMELON.

A nearly mature watermelon was inoculated by puncture with conidia from a bean culture. No signs of infection followed.

It will be observed that no successful inoculations from bean to cucurbits were made. These results agree with those of Edgerton (30) but not with those of Halsted (39).

# CAMELLIA TO BEAN.

On June 4 five young pods of bean grown in the greenhouse were inoculated by surface application of conidia in sterile water. No signs of infection followed.

On June 5 the same experiment was repeated with six pods, but without success.

#### CARYOTA TO COTTON.

On April 1 stigmas of three cotton flowers were inoculated by applying conidia in sterile water to the surface. The young bolls soon dropped, but when placed in moist chamber no conidia developed.

# CHERIMOYA TO APPLE.

On October 2 three apples were inoculated by puncture with conidia from a culture. Decay followed about the points of inoculation in one week and acervuli were produced.

#### CHERIMOYA TO GRAPE.

On October 4 eight mature berries were inoculated by puncture with conidia from a culture. At the end of two weeks all the grapes were decayed and seven showed acervuli.

#### CINNAMON TO COTTON.

On April 1 three flowers were inoculated by the application of conidia in sterile water to the stigmas. No signs of infection followed, but the young bolls soon fell off. These bolls were kept in moist chamber for several weeks, but no conidia or acervuli developed.

# COTTON TO APPLE.

Mature fruit was inoculated by puncture with conidia from a pure culture of the cotton fungus. A slight decay appeared in two weeks and a few acervuli with setæ bearing spores were found. At the end of a month the decayed spots had become larger and colored sporophores were also found mixed with the setæ.

On October 6 four apples were inoculated by applying conidia from cotton cultures to the surface. At the end of three weeks there

was no sign of infection or decay.

On July 25 four apples were inoculated by puncture with conidia from a cotton boll. A slight decay appeared on two at the end of a month. At the end of one and one-half months the spots were three-fourths of an inch in diameter. Cultures made from the pulp from these spots produced acervuli and conidia with setæ, also brown septate conidia, such as are mentioned by Clinton (19). Two apples inoculated later in the same way produced practically the same result. The development of rot was very slow.

On October 6 four apples were inoculated by puncture using conidia from cultures made from the apple which was originally inoculated from cotton. Three of these apples showed small rotten spots at the end of 8 days. At the end of 20 days all showed rot and two produced acervuli. No typical setæ were found, but some brownish sporophores occurred which were rather intermediate in form between sporophores and setæ. This second generation of the cotton fungus on the apple developed much more rapidly than the first generation and indicated the possibility of its soon developing fully as fast as the form from the apple. This experiment and similar ones with other hosts appear to indicate that these organisms under certain conditions may rather quickly adapt themselves to different hosts though retaining their specific morphological characters. These results also suggest the possibility of the apple acting as a bridging host in some instances. This is in accord with the work of Salmon (68) on mildews and with that of Ward (94) on rusts.

#### COTTON TO BEAN.

On June 4 six young pods from a greenhouse plant were inoculated by applying conidia in sterile water from a cotton boll to the surface. No signs of infection ever appeared.

#### COTTON TO COTTON.

On March 19 five flowers were inoculated by applying conidia in sterile water from a cotton boll to the stigmas. All the bolls soon turned black and nearly all dropped off. One boll that remained on the plant about two weeks before it died became half covered with acervuli.

On June 24 six young bolls were inoculated by puncture with conidia from a cotton boll. In about two weeks small, dark, sunken spots had formed on all and four showed acervuli.

# COTTON TO PUMPKIN.

On November 2 a pumpkin was inoculated by puncture with conidia from a pure culture of the cotton fungus. No signs of infection ever appeared.

# COTTON TO HUBBARD SQUASH.

A squash was inoculated by puncture with conidia from a pure culture. No signs of infection ever appeared.

# COTTON TO WATERMELON.

On November 2 a watermelon was inoculated by puncture with conidia from a pure culture from cotton. No rot followed, but a slight development of hyphæ and a few acervuli formed at the puncture. Spore-bearing setæ were present in these acervuli, as is usually the case with Glomerella gossypii.

# CRANBERRY TO APPLE.

On April 27 six sound apples were inoculated by puncture with conidia from a pure culture. No rot or acervuli had developed at the end of a month. Later inoculations produced a slight decay in one instance and a few acervuli formed.

# CRANBERRY TO SWEET PEA.

On April 6 stems and young leaves of young plants in the greenhouse were inoculated with ascospores in sterile water applied to the surface. No signs of infection ever appeared.

#### CURCULIGO TO COTTON.

On April 2 three flowers in greenhouse were inoculated by applying conidia in sterile water to the stigmas. No signs of infection appeared, but the young bolls dropped off. They were placed in a moist chamber, but no acervuli developed.

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#### DEWBERRY TO APPLE.

On October 2 three apples were inoculated by puncture with conidia from a culture from dewberry. At the end of a month only one apple showed a decayed spot, 1.75 inches in diameter. Small decayed spots occurred on the other two apples at points of inoculation 26 days later.

On October 12 four Willow Twig apples were inoculated by puncture with conidia from a culture. At the end of three weeks only one fruit showed development of rot, about 1.75 inches in diameter.

On October 26 three apples were inoculated by puncture with conidia from culture from the apple previously inoculated from dewberry. Only a slight decay occurred about the point of inoculation after several weeks and no acervuli formed. Cultures made from these decayed spots produced acervuli.

#### DEWBERRY TO APPLE TO AGAR TO APPLE.

On December 7 three apples were inoculated by puncture with conidia from an agar culture made from the first apples inoculated from the dewberry. Infection followed in all cases. Decayed spots 0.5 to 0.75 inch in diameter developed in six days, but no acceptable acceptable.

#### DEWBERRY TO APPLE TO APPLE TO AGAR TO APPLE.

On January 3 three apples were inoculated by puncture with conidia from a pure culture made from the second generation of the dewberry fungus on apples. Infection followed in all cases. The decayed spots developed more rapidly than in the previous generation and abundant accrvuli were formed.

On January 10 three apples were inoculated by puncture with conidia from a culture from the second generation grown on apple. Infection followed in all cases. Decayed spots an inch in diameter developed in 10 days and numerous acervuli appeared. These experiments seem to indicate that the dewberry form of Glomerella becomes quickly adapted to growth on apples, developing in the third generation about as rapidly as the fungus taken directly from apples and producing typical rot and acervuli.

#### DEWBERRY TO GRAPE.

On October 16 eight berries were inoculated by puncture with conidia from a culture from the same strain of the dewberry Gloeosporium used in the inoculations with apples. At the end of two weeks some rot was found, but no acervuli were ever produced.

#### FIG TO APPLE.

On September 14 feur apples inoculated by puncture with conidia from a pure culture from the fig produced decay of the usual appearance of bitter rot, and acceptual were formed.

On October 2 three apples were inoculated by puncture with conidia from a pure culture from the fig. Infection followed in all cases and decay developed as rapidly as in inoculations made directly from apple to apple and typical acervuli also developed as shown in Plate XVI, figure 7.

# FIG TO FIG.

Eight nearly mature figs were inoculated, four by puncture with conidia from cultures and four by application of the same to the unbroken surface of the fruit. Rot developed in all cases and acervuli formed. There is some doubt about this experiment, however, on account of the fact that three out of the seven checks used also developed rot.

#### FIG TO GRAPE.

Eight mature berries were inoculated by puncture with conidia from a pure culture from the fig. Most of the berries were rotten at the end of three weeks and three showed acervuli.

#### FICUS LONGIFOLIA TO RUBBER PLANT.

The under side of a young leaf attached to a plant was inoculated by placing conidia from a pure culture in sterile water on the uninjured surface and covering the spot with a Van Tiegham cell. At the end of 24 days there was no sign of infection. The leaf was then removed from the plant and placed in a moist chamber. Acervuli developed on the inoculated spot after two weeks but not on the check spot covered in the same way.

### GOOSEBERRY TO APPLE.

On December 7 three apples were inoculated by puncture using hyphæ and probably ascospores from a pure culture containing masses of mature perithecia. On December 15 two of the apples showed rotten spots 1 cm. in diameter at the points of inoculation and the third a discoloration of the skin. By January 7 the first two apples were half rotten and the third about one-third decayed, and two of them produced acervuli. Cultures made from these apples produced both acervuli and perithecia of the fungus, the perithecia predominating.

#### GRAPE TO APPLE.

In four different experiments in which apples were inoculated by puncture with conidia from cultures from grapes or direct from the host typical bitter-rot followed and acervuli developed.

On August 19 eight Smokehouse apples were inoculated with conidia from rotten grapes from another source. This strain of the fungus appeared to be weak or not adapted to development on apples. Rot developed on only two of the inoculated fruits. The spots were small and increased very slowly and no acervuli were produced. Cultures were made from these spots.

#### GRAPE TO APPLE TO AGAR TO APPLE.

On August 24 six Smokehouse apples were inoculated by puncture with conidia from the culture made from apples inoculated from grape as mentioned above. Decay developed very slowly about the point of inoculation but a little faster than in generation 1. Finally some acervuli were produced on all but one of the apples.

On September 18 the above experiment was repeated with six Smokehouse apples. The development of rot this time was somewhat faster than before and acervuli developed on all.

# GRAPE TO AGAR TO APPLE TO AGAR TO APPLE TO APPLE.

Four apples were inoculated by puncture with conidia from an apple inoculated with the third generation of the fungus from the grape. Rot developed about as rapidly at the point of inoculation on these fruits as is usual in transfers directly from apple to apple and at the end of 8 days acervuli were found.

# GRAPE TO AGAR TO APPLE TO GRAPE.

On September 23 twelveripe berries were inoculated by puncture with conidia from an apple. Only four developed the usual rot followed by acervuli. The others softened somewhat but no acervuli formed.

#### GRAPE TO AGAR TO GRAPE.

On September 23 twelve ripe berries were inoculated by puncture with conidia from a culture from a grape. At the end of two weeks five were rotten and acervuli had formed. Seven of these eventually decayed and showed acervuli.

#### GRAPE TO AGAR TO APPLE TO TOMATO.

On September 29 four green tomatoes were inoculated by puncture with conidia from a culture from a grape. Slight decay followed in only one case, but perhaps not from the inoculation. No acervuli were ever produced.

### GRAPE TO PUMPKIN.

A pumpkin was inoculated by puncture with conidia from a culture from a grape. Rot developed rapidly and acervuli formed.

# GRAPE TO HUBBARD SQUASH.

A nearly mature squash was inoculated by puncture with conidia from a culture from a grape. Rot developed very slowly at one point of inoculation only. A few acervuli were found at the end of a month.

#### GRAPE TO WATERMELON.

A nearly mature watermelon was inoculated with conidia by puncture from a culture from a grape. Rot developed rapidly at the point of inoculation and numerous acervuli formed.

# GUAVA TO APPLE.

Two apples were inoculated by puncture with conidia from a culture from leaves of guava. Rot developed at the points of inoculation about as rapidly as in the case of transfers from apple to apple. Acervuli were present at the end of a week. No setæ were found.

# GUAVA TO BEAN.

Six young pods of a wax bean from the greenhouse were inoculated by surface application of conidia from guava. No signs of infection ever followed.

## GUAVA TO COTTON.

Three flowers of a cotton plant from the greenhouse were inoculated by applying conidia from guava to the surface of the pistil. No signs of infection were found at the end of two weeks, but two of the small bolls which developed from the inoculated flowers developed acervuli with setæ when placed in a moist chamber.

#### GUAVA TO PUMPKIN.

A pumpkin was inoculated by puncture with conidia from a culture from guava leaves. Rot developed rapidly and acervuli were produced in abundance.

# GUAVA TO HUBBARD SQUASH.

A squash was inoculated by puncture with conidia from a culture from guava leaves. No rot appeared and no acervuli developed.

#### GUAVA TO WATERMELON.

A nearly mature watermelon was inoculated by puncture with conidia from a culture from guava leaves. Rot developed rather rapidly. Numerous acervuli were produced, as shown in Plate XVIII.

# LEMON TO APPLE.

On September 14 four Willow Twig apples were inoculated by puncture with conidia from a culture from a lemon. Rot of the usual appearance developed rapidly in all cases and typical acervuli formed.

On October 2 three apples were inoculated by puncture with conidia from a culture. Rot developed in all cases. The spots were 2 centimeters in diameter at the end of a week and acervuli were present.

#### LEMON TO CABBAGE.

On December 17 leaves of a young cabbage plant in the greenhouse were inoculated by puncture with conidia from a lemon leaf. No signs of infection ever followed.

#### LEMON TO COTTON.

On December 17 leaves and bolls were inoculated by puncture with conidia from lemon leaves. No signs of infection were found at the end of a month.

#### LEMON TO CRANBERRY.

On December 17 leaves of a plant from the greenhouse were inoculated by puncture with conidia from a lemon leaf. No signs of infection were ever seen.

#### LEMON TO GRAPE.

On October 14 eight mature berries of Niagara grapes were inoculated by puncture with conidia from a culture. At the end of two weeks most of the berries were rotten and three bore acervuli.

#### LEMON TO ORANGE.

On December 17 orange leaves were inoculated by puncture with conidia from a lemon leaf. No signs of infection ever followed.

#### LEMON TO RUBBER PLANT.

On December 17 leaves on a living plant were inoculated by puncture with conidia from a lemon leaf. No signs of infection ever followed.

#### LEMON TO TEA.

On December 17 leaves of a living plant were inoculated by puncture with conidia from a lemon leaf. No signs of infection were found at the end of a month.

#### LOQUAT TO BEAN.

On June 4 six young pods from a greenhouse plant were inoculated by immersion in sterile water containing conidia. No signs of infection ever followed.

#### MANDARIN TO BEAN.

On June 4 six young pods from a greenhouse plant were inoculated by immersing beans in sterile water containing conidia. No signs of infection ever appeared.

#### MANDARIN TO COTTON.

On April 1 flowers of a greenhouse plant were inoculated by applying conidia to the stigmas. No signs of infection followed, but the young bolls soon dropped off. They were placed in a moist chamber, but no Gloeosporium developed.

# MARANTA TO COTTON.

Stigmas of three flowers of a greenhouse plant were inoculated in the same manner as above. No signs of infection followed, but the young bolls dropped off. They were placed in a moist chamber, but no Gloeosporium developed.

## ORANGE TO BEAN.

On June 5 six young pods on a greenhouse plant were inoculated by immersion in sterile water with conidia. No signs of infection ever appeared.

#### ORANGE TO COTTON.

On April 1 three flowers were inoculated by applying conidia to the stigmas. No indications of infection appeared, but the young bolls soon dropped. These bolls were kept in a moist chamber, but no Gloeosporium developed.

On June 24 five young bolls were inoculated by puncture with conidia from this host. Two developed slight decay and when removed to a moist chamber acervuli developed. Six others inoculated in the same way a little later showed scarcely any signs of decay about the point of inoculation but developed acervuli when removed to a moist chamber.

## ORANGE TO RUBBER PLANT.

On February 4 two leaves of a small plant in the laboratory were inoculated by making a slight incision across the midrib near the tip and immersing the leaves in sterile water containing conidia from a culture. After five weeks no signs of infection were apparent. The leaf was removed from the plant, its surface thoroughly washed with corrosive-sublimate solution, and placed in a moist chamber. Twelve days afterwards, the leaf showed abundant acervuli extending about 3 inches from the tip. The two checks treated in the same manner throughout produced no Gloeosporium when kept in a moist chamber.

# ORANGE TO TEA.

On February 4 two leaves of a growing plant in the laboratory were inoculated by making a slight incision in the midrib and immersing the leaf in sterile water containing conidia. A check was also treated in the same manner but not inoculated. The inoculated leaf developed immature acervuli on a small dead area surrounding the puncture while still attached to the plant. The check leaf was removed and placed in a moist chamber, but no Gloeosporium developed. Another leaf, however, on the same plant which appeared healthy was removed and placed in a moist chamber and this developed abundant acervuli, which indicates that it is practically impossible to determine whether or not a leaf or any portion of a plant already contains the fungus in a dormant condition. Results of experiments of this kind are thus shown to be somewhat uncertain.

# PEPPERWORT TO COTTON.

Stigmas of two cotton flowers from the greenhouse were inoculated by the application of conidia in sterile water. The young bolls soon dropped off. They were kept in a moist chamber but no Gloeosporium developed.

#### PHORMIUM TO CABBAGE.

Five young plants in pots in the laboratory were sprayed with distilled water containing conidia and then covered with a bell jar. Leaves of both inoculated plants and checks soon turned yellow and dropped off. All these leaves were placed in moist chambers. Acervuli of Gloeosporium appeared on the inoculated leaves but not on the check. In two cases acervuli were produced on the inoculated leaves while still attached to the plant.

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#### PITCAIRNIA TO BEAN.

Six young pods on a plant in the greenhouse were inoculated by immersion in sterile water containing conidia. No signs of infection ever appeared.

### PITCAIRNIA TO COTTON.

The stigmas of two flowers of a greenhouse plant were inoculated with conidia in sterile water. The young bolls soon dropped off. These were placed in a moist chamber, but no Gloeosporium ever developed.

# POMELO TO APPLE.

Three apples were inoculated by puncture with conidia from a culture. Infection followed in all cases. Spots 2 centimeters in diameter developed at the end of a week and acervuli formed.

## POMELO TO GRAPE.

Eight mature berries of a Niagara grape were inoculated by puncture with conidia from a culture. At the end of two weeks the berries were mostly rotten, and acervuli were found on six of them.

#### POMELO TO POMELO.

Two sound fruits were inoculated by applying conidia in sterile water to the uninjured surface. The conidia germinated, and a slight development of mycelium with scattered conidia and chlamy-dospores appeared on the surface but the tissue beneath remained sound. No rot developed. In another case the same experiment was tried, applying the spores to the surface which had been injured by red spiders. The results were exactly the same as in the case just mentioned. No rot developed.

#### PRIVET TO BEAN.

Six young pods from a greenhouse plant were inoculated by immersion in sterile water containing conidia. No signs of infection ever appeared.

# RUBBER PLANT TO FIG.

Four figs were inoculated by puncture with conidia from a leaf. Rot developed in all cases. Four other fruits were inoculated by application of conidia in sterile water to the uninjured surface of the figs. Rot developed but did not appear to start immediately at the point of application of the spores and as one of the checks in this experiment also developed rot the results are uncertain, Both lots of figs are shown in Plate XVII.

# INOCULATION EXPERIMENTS BY OTHER INVESTIGATORS.

In order to compare and render convenient and accessible the complete results of experimental inoculations with Glomerella and its conidial forms up to the present time, the published data of other investigators have been arranged in tabular form. Where particular facts are wanting they are not given by the authors cited.

Table IV.—Results of inoculations as reported by other investigators.

Host.						Ti!	
Original.	Inoculated.	Part inoculated.	Method of inoculation.	Material used. <sup>1</sup>	Source of material.	Result.	Experi- menter.
Apple	Apple	Green fruit	Puncture .				Clinton, 1902.
Do	do	FruitGreen fruit	Surface	A		do	Do. Do.
Do	do	do	ao	C		Failure	Do.
Do	do	Fruit on tree Fruit	do	Α		Success.	Do. Do.
Do	do	do	do	C		do	Hasselbring.
Do	ofi	đo	do	C	Canker Pure culture	do	1906. Scott, 1906.
Do	do	Branches	Puncture	A	Pure culture	do	Spaulding and Von
				_			Schrenk, 1903.
Do	do	Fruit	do	C	do	do	Do. Do.
Do	do	do	do	C	Canker	do	Do.
	do	Ripe fruit				do	Edgerton, 1911.
	do						Taubenhaus, 1911.
Do Do		Ripe fruit Fruit	Surface	C	Hogt	do	Clinton, 1902. Cobb, 1904.
Do	do	do	do	C. or H	Hostdo	do	Halsted, 1892.
Do	Borborer	do	Surface	C	do	Failure	Hasselbring.
							1906.
Do Do	Bean Bush bean	Pods on vine	Puncture . Surface	C	Host	Failure	Halsted, 1892. Taubenhaus,
Do	Cherry	Fruit	Puncture .	C	Host	Success	1911. Cobb. 1892
Do	do	do	do	C	do	do	Cobb, 1892. Cobb, 1904.
Do		do		£ 2	do	do l	Halsted,1892. Cobb, 1904.
Do	Hawthorn	do	do	č <u>.</u>	do	do	Do.
Do Do	Lemon Lima bean	Pods on vine	do	C. or H	do	do	Halsted, 1892. Taubenhaus,
Do	Mango	Fruit		C	Host	do	1911. Cobb, 1892.
Do	Nectarine	do	do	C	do	do	Cobb, 1904. Cobb, 1892. Cobb, 1904.
Do	do	do	do	C	ldo	do	Cobb, 1904.
Do	Peach	Green fruit	do	C		do	Clinton, 1902.
Do	do	do	Surface	C		do	Do.
Do	do	Fruit	Puncture.	C	Hostdododo	do	Cobb, 1892. Cobb, 1904.
Do	do	doBranch	do	C. or H	do	do	Halsted, 1892.
Do	Pear	Branch	Puncture		do	D011b13131	Burrill, 1907.
Do	Pear do do do	Green fruit.	do	č		do	Clinton, 1902.
Do	do	Ripe fruit	Surface	Ç	Host	Doubtful	Do.
Do	do do	do	do	č	Hostdo	success.	Cobb, 1892. Cobb, 1904.
ДО	do		ldo	C	do	do	Halsted, 1892.
							Taubenhaus,
Do	Pepper	Fruit	do	C. or H.	Host	do	Cobb, 1904.
Do	Persimmon .	do	do	o. or H.	Hostdo	do	Halsted, 1892. Do.
Do	Plum	do	do	C	Host	30	Cobb, 1892.

¹ A.=ascospores; C.=conidia; H.=hyphæ.

Table IV.—Results of inoculations as reported by other investigators—Continued.

	Host,						
			Method of	Material	Source of		Transmi
Original.	Inoculated.	Part inoculated.	inoculation.	used.	material.	Result.	Experi- menter.
Apple	Plum to to-	Fruit	Puncture.	C	Host	Success.	Cobb, 1904.
Do	Quincedo	Green fruit Fruit	do	C	Host	Failure . Success .	Clinton, 1902. Cobb, 1904.
Do	Sweet pea	Young leaves	Surface	C. or H. C.	Culture	do	Halsted, 1892. Edgerton, 1908.
Do	do	Seedlings	do	C	Host	do	Sheldon, 1905.
Do	do	do	Puncture . Surface	C		do	Taubenhaus, 1911. Do.
Do	Tomatodo	Green fruit. Fruit.	do	C. C. C. or H.	Host	do	Clinton, 1902. Cobb, 1904. Halsted, 1892.
Asclepias	Apple	do	do	C. or H		Doubt- ful.	Halsted, 1892. Edgerton, 1908.
Avocado	Pear	Fruit on tree	do	C		Rot, but	Taubenhaus, 1911.
Do	Sweet pea	Seedlings	Surface	C		typi- cal. Failure	Do.
Do Banana	Apple	Fruit	Puncture	C	Host	do	Do. Cobb, 1904.
Do	do	do		0		do	Laubert, 1910.
Do	Lemon	do	Puncture	C	do	Success . Doubt- ful.	Cobb, 1904. Do.
Do	Passion fruit	do	do	C	do	Very	Do. Do.
D.	Dooch	a.	a.	C	a.	doubt-	Do.
Do	Peach	do	do	C	do	Success.	Do.
Bean	Quince	Stem and leaves.	Surface	č	do	Failure .	Do. Edgerton, 1910.
Do	Apple	Fruit		C		do	Edgerton, 1908.
Do	do	Ripe fruit		C	Pure culture	do	Edgerton, 1911.
Do	Bean	Leaves	Surface	C	Culture	Success.	Edgerton,
Do	dodo. Citron 2	Stems	do Puncture .	C	dodo Host	do	Do. Do. Halsted,1893.
Do Do	Cotton	Fruit	Surface	C	ELUSE	Failure	Edgerton, 1910.
Do	Cucumber	Leaves, stems, fruit.	do	C	Treat	do	Do.
Do	Eggplant Pea	Fruit Leaves	Puncture . Surface	C. or H		Success . Failure	Halsted, 1892. Edgerton, 1910.
Do Do	Pear Pepper	Fruit	Puncture .	C. or H	Hostdo	Successdo	Halsted, 1892. Do.
Do Clover	Persimmon . Apple	Ripe fruit	do	do C	Pure culture	Failure .	Do. Edgerton, 1911.
Coffee	do	Fruit		C		Success.	Edgerton, 1908.
CCotton	FigAppledo	Leaves Fruit Ripe fruit		C	Pure culture	do do <sup>3</sup> Failure .	Do. Do. Edgerton, 1911.
Do	Bean	Seed	Surface	C		do	1911. Edgerton, 1910.
Do	Cotton	Very young bolls.	do	C	Culture	(4)	Barre, 1909.
Do	do	Stems Leaves	Puncture .	C		Failuredo	Do. Do.
Do	dodoFig.	Petioles	Surface	C	Thomas 14	Success .	Do. Do.
Do	Fig	Green fruit		C	Pure culture	Partial success.	Edgerton, 1911.

<sup>1</sup> Development slow.
2 Citrullus vulgaris var.
3 Takes poorly.

<sup>4 60</sup> per cent infected. • 37 per cent infected.

 ${\tt Table\ IV.--} Results\ of\ inoculations\ as\ reported\ by\ other\ investigators{--} {\tt Continued}.$ 

Host.		Method of	Material	Source of	Result.	Experi-	
Original.	Inoculated.	Part inocu- lated.	inoculation.	used.	material.	Resuit.	menter.
Dracaena	Apple	Fruit		C		Success.	Edgerton,
Do Eggplant	Fig Bean Pepper	Leaves Pods? Fruit	Puncture .	C. or H	Host	do	Do. Halsted,1892. Do.
Do	Apple	Ripe fruit		C	Pure culture	do	Edgerton,
Do	Bean	Seeds, stems, leaves. Green fruit	Surface	C	Pure culture	Failure .	Edgerton, 1910. Edgerton,
Do	Figdo	do		C	Culturefrom	do	1911. Do.
Guava	Apple	Fruit	Puncture	C	branch. Host Culture	Success	Cobb, 1904. Sheldon,1906.
Do	do	Picked fruit Unpicked fruit.	do	C	do	do	Do.
Do	do	Young un- picked fruit.	do	C		Failure	Do.
Do	do	Picked fruit. Twigs	do	C		Success Failure	Do. Do.
Do Do	Banana Bush bean	Fruit Pods on vine	do	C	Host	Successdo	Cobb, 1904. Taubenhaus,
Do	Damson	Fruit	do	C	Culture	do	1911. Sheldon,1906.
Do	plum. Apple to hawthorn.	do		C	Host	do	Cobb, 1904.
Do Do	Passion vine Peardo	do do	dodododo	C C	do do	Failure . Success . Very	Do. Do. Do.
Do	do	Fruit on tree		C		doubtful. Rot, but not typ-	Taubenhaus,
Do Do	Quince Quince to guava.	Fruitdo	do	C	Hostdo	ical. Successdo	Cobb, 1904. Do.
Do	Sweet pea	Seedling		C		Failure .	Taubenhaus, 1911.
Do Kentia Mandrake	Appledo	Green fruit Fruit	Surface Puncture .	C	Host	Success .	Do. Cobb, 1904. Taubenhaus,
Do	Lima bean Pear	Pods on vine	Puncture.	C	*	do	1911. Do. Do.
Do	Sweet pea	Seedling Seedling	Surface	Ç		do	Do. Do.
Oak gall Do Do	Apple Lima bean Pear	Fruit Pods on vine Fruit	Puncture.	C		do	Do. Do. Do.
Do	sweet pea	Seedling	Surface	l C		do Doubt-	Do. Do.
Orange Passion vine	Appledo	Fruitdo		c	Host	Doubt- ful. Failure	Edgerton, 1908. Cobb, 1904.
Do	Date to	do	do	C	do	do	D0.
Do Do	Guava Peach Pear	do do		C	do	do	Do. Do. Do.
Passion leaves Passion vine	Pepper	do	do	C	dodododo	Success . Failure	Do. Do. Do.
Do	Pineapple	do	do	C	do	-J	Do. Do.
Pear. Do	Banana	dododododododo.	do	C. or H.	do	Failure Successdo	Do. Halsted,1892. Do.
Do	Lemon Pepper	do	do	do		do	Do. Do.
Pepper	Apple	do	do	C. or H.	Host	do	Do. Cobb. 1904.
Do	do	Ripe fruit		C	Pure culture	do	Halsted, 1892. Edgerton, 1911.

Table IV.—Results of inoculations as reported by other investigators—Continued.

		+ nappetition-					
Host.							
			Method of	Material	Source of		Experi-
		Part inocu-	inoculation.	used.	material.	Result.	menter.
Original.	Inoculated.	lated.					
		interest.					
_							
Pepper	Banana	Fruit Stem and	Puncture.	C. or H		Success.	Halsted, 1892.
Do	Bean	leaves.	Surface	C		Failure.	Edgerton, 1910.
Do	Fig.	Pod	Puncture .	C. or H .		Success .	Halsted. 1892.
Do	Fig	Green fruit		C	Pure culture	do	Edgerton, 1911.
Do	Pear	Fruit	Puncture .	C		do	Halsted,1892.
Do	Pear Persimmon.	Ripe fruit	do	Č		do	Do.
Poplar	Apple	Ripe fruit		C	Pure culture	Partial	Edgerton, 1911. Cobb, 1904.
Quince	do	Fruit	Puncture .	C	Host	Success.	1911. Cobb 1904
Raspberry	do	do	- unovaio .	Č		do	Edgerton,
			~ .		G 4:		l 1908.
Rose	Bean	Picked pods.	Surface	C	Culture	Failure.	Edgerton, 1910.
Rubber plant.	Rubber plant	Detached	do	A	do	Success .	Koorders,
		leaves.			}		1907.
Do	do	Seedling leaves.	do	A	do	do	Do.
Sarracinia	Fig	Leaves		C		do	Edgerton,
							1908.
Sassafras	Apple	Fruit		C		do	Taubenhaus,
Do	Lima bean	Pods on vine	Puncture .	C		do	1911. Do.
Do	Pear	Fruit	do	C		do	Do. Do.
Do	Sweet pea	Seedling	do	C		do	Do.
Do	do	seeding	Surface	C		do	Do. Do.
Silver maple .	Apple	SeedlingdoRipe fruit		C	Pure culture	do	Edgerton, 1911.
T) a	Fig			C	do	do	1911. Do.
Sweet pea	Fig Apple	Green fruit Fruit		Č	do	do	Taubenhaus,
- Direct postiti							1911.
Do	Bush bean	Fruit on tree	Puncture.	C		do	Do. Do.
Do	bush bean	Pods on vine	Surface	Č		Failure.	Do.
D0	Lima bean.	do	Puncture.	Ç		Success.	Do.
Tomato	Apple	do	do	C. or H		do	Halsted, 1892 Do.
Do	Bean	Pod	do	do		do	Do.
Do	Eggnlant .	Ermit	i do				Do.
Do	Pennor	do	do	ao	Culture	do	Do. Chester, 1893.
Do	do	UV	1 UU	V		do	Do.
Do	do	do	Surface	C		Failure	Do.
Do	Pepperto	do	Puncture.	C		Success .	Do.
Do	pepper. Pepper to	do	do	C		Failure .	Do.
	squash.	3	,	C		Consessed	De
Do	Pepper to tomato.	do	ao	U	************	Success .	Do.
Do	Tomato	do	Surface	C		Failure	Do.
Do	Quince	do	Puncture.	C		Success . Failure	Halsted, 1892.
Watermelon	Apple	do		C	*************		Edgerton, 1908.
Do	Bean	do	Puncture.	C. or H	Hostdododo	Success .	Halsted, 1893.
Do	Citron 1	Picked fruit.	do	do	Host	do	Do. Sheldon,1904.
Do	Cucumber	Leaves Unpicked	Surface Puncture.	C	do	do	Do.
D0		fruit.					
Do	do	Picked fruit.	Surface	C	Culture	do	Do. Do.
Do	Gourd Muskmelon.	Seedling Unpicked	Puncture.	C		do	Do.
		fruit.		~			Dr
Do	do	Seedling	Surface	C	Culture	do	Do. Do.
Do Do	Pumpkin	do	do	C	Culture	Failure	Do.
Do	Pear	Fruit on tree	Puncture .	Č	************	Sucess	Taubenhaus,
				C		do	1911. Do.
Do	Sweet pea	Seedlingdo	Surface	C		do	Do.
Yellow sweet	Apple	Ripe fruit		C	Pure culture	Failure .	Edgerton,
clover.		_		C	1	Partial	1911. Do.
Do	Fig	Green fruit		0	stem.	success.	

# PARASITISM OF GLOMERELLA.

That Glomerella is an active parasite in certain races and under certain conditions would never be questioned by one who has had an opportunity to observe a severe attack of bitter-rot of the apple such as occurred in an orchard near Vienna, Va., in 1911. Several trees of Willow Twig apples well cared for and in a thrifty condition and bearing a good crop of fruit were attacked in July when the fruit was about half grown. Within a week or two nearly every apple on these trees showed bitter-rot spots which developed rapidly and soon destroyed the crop. This result was apparently due to a combination of favorable factors, the presence of a virulent race of the fungus, a susceptible variety of fruit (other varieties immediately adjoining showed little or no bitter-rot), and optimum conditions of temperature and moisture for infection and development.

Many races of Glomerella under ordinary conditions appear to be rather weak parasites. The different degrees and manifestations of parasitism among the fungi are so numerous and varied that it is very difficult to classify them satisfactorily in this respect. The possession of dormant hibernating hyphæ which infest the seeds of some annual plants, as in the case of the cotton and bean anthracnoses, the shoots of perennials, as in orange, and the leaves of evergreens, as in the case of the rubber plant, coffee, tea, citrus fruits, and other subtropical plants, indicates a considerable degree of specialization which would appear to give Glomerella a higher rank among parasites than the Fusarium forms of Nectria and closely related organisms. On the other hand, its ready growth on artificial culture media. dead organic matter and matured fruits suggests a low form of parasitism. As already mentioned, Appel and Wollenweber (1) have suggested that the fact that an organism will complete its life history on artificial culture media or dead organic matter is an indication that it is a saprophyte. As has already been pointed out, this suggestion does not seem to accord with the known facts.

The majority of the inoculation experiments which have been reported were performed by puncture of mature or nearly mature fruits. This method does not furnish the best proof of the parasitism of the organism. It is quite possible that the fungus would grow and develop rot under such conditions but still might not under natural conditions be able to gain entrance through the unbroken surface of the fruits.

The experiments of Edgerton (28), Rolfs (66), Barre (7,8), Hasselbring (45), Bessey (13) and others, as well as some of those of the present writers, have shown that cross-inoculations can be successfully made by the application of spores to the uninjured stems, leaves, and flowers of living plants. Hasselbring (45) has shown a way in

which the appressoria, or chlamydospores, assist the fungus in gaining entrance to the tissues of the host. In the majority of cases the most probable explanation of the dormant infections which have been shown to be present in so many instances in leaves and fruit showing no external evidence of disease, is that the conidia or ascospores germinate whenever they come in contact with the plant surface under favorable conditions of temperature and moisture and produce appressoria, which are able to endure more unfavorable conditions than the spores and which in turn send a germ tube through the epidermis. This tube apparently penetrates at first but a very short distance and does little harm to the host cells, remaining in a dormant or inactive condition until the host becomes weakened or injured or the organ infected dies a natural death. Bodies resembling appressoria have been found on the surface of normal apple leaves upon which the fungus developed in a moist chamber and they are sometimes found in abundance on the surface of lemons and other citrus fruits. It is difficult positively to identify these bodies on a leaf surface and trace the germ tube in the tissue, and the writers have as vet been unable to devote the necessary time to this feature of the investigation to verify conclusively the suggested explanation of the facts observed.

Large series of microtome sections of presumably infected leaves, the unused portions of which developed the fungus when placed in a moist chamber, have been studied, but the presence of fungus hyphæ has not been demonstrated with certainty. This would be quite natural if the supposition that the dormant infection is restricted to a short hypha or germ tube just penetrating the surface is correct. The experiments with leaves in moist chamber, especially deciduous leaves, show that the discoloration of the tissue and the development of the fungus start at rather definite points on the leaves and spread from these in a more or less circular manner, as shown in Plate V. In the case of the citrus fruits there appears to be quite clear evidence, from the work of Rolfs (67), Bessey (12, 13), as well as from that of the writers with leaves and shoots in moist chambers that the fungus in some cases enters the stem by way of the flowers and works back through the tissue. The development of the fungus first in the petiole and along the midrib, as frequently happens in the case of orange and rubber plant leaves in moist chamber, would suggest this possibility, though in these latter cases the infection may have been localized on the petiole or midrib.

These organisms generally develop most rapidly and do most damage to nearly or quite mature fruit and have developed special methods of insuring their survival and distribution from season to season, not only by means of appressoria but by hibernating hyphæ in old

fruits, leaves, and branches and by ascospores.

# DIFFERENCES IN VIRILITY OF RACES FROM THE SAME HOST.

From the experiments of the writers it appears clear that there is considerable variation in the virility of different races or strains of Glomerella just as there is in their morphological characters. As mentioned in the records of inoculation experiments, conidia from cultures from different apples when inserted in the fruit showed considerable difference in the production of rot. Some of these races produced rot more slowly than those derived from other hosts. This fact increases the difficulty of developing a resistant variety of any host as a means of counteracting disease, as has been attempted with cotton and bean. Barrus (9) at first reported certain varieties of beans as entirely resistant to the anthracnose fungus with which he was working. Later he (10) found that when other strains of the fungus were used these varieties became infected with the disease. The observations of Scott (73) that the Ben Davis apple is very severely attacked by bitter-rot in Arkansas, while it is seldom seriously injured by the disease in Virginia, although other varieties in the same locality are destroyed, is also perhaps to be accounted for by the presence of strains of different virility in the two regions. A variety or race of a host may be very resistant to a certain strain of a fungus but may succumb to a more virile strain of the parasite at any time. This also accords with the results of animal pathologists as reported by Slack (82) and others in the case of pathogenic bacteria. We must not fail to recognize that the parasite is apparently capable of as great variability in every direction as the host.

# METHODS OF PREVENTION AND CONTROL.

In the cases in which these diseases are known to be transmitted through the seed, as in cotton and beans, the trouble may be largely prevented by the careful selection of fungus-free seed, as shown by Barre (7) for cotton and Whetzel (95) for beans. The work of Duggar (27) and Barre (7) with cotton, Barrus (9, 10) with beans, and Bain and Essary (4) with clover seems to indicate that more or less resistant varieties may be obtained. In this connection it should be constantly borne in mind, as already stated, that the fungous parasite is an organism evidently subject to the same laws and possesses the same or possibly greater capacity for variation, not only in form but in virility, as its host plants. This has been demonstrated by the writers' work, as indicated elsewhere in this paper, and is confirmed by the experience of Barrus (10) and others. Sanitary measures and eradication methods will prove helpful. Cutting out and destruction of cankers where they occur, the destruction or burying through cultivation of mummied fruits and infested leaves, should be practiced wherever practicable.

Spraying is, however, the most important general method of preventing disease caused by Glomerella. The work of Scott (73) and others with the bitter-rot of apples has shown that the proper application of Bordeaux mixture will largely protect fruit from this disease. Rolfs (66) has shown that the wither tip of citrus fruits, due to Glomerella, can also be controlled by spraying. There is no reason to doubt that diseases of other plants caused by the same fungus can be largely prevented by the proper use of Bordeaux mixture. In determining dates for spraying it is important to first discover the natural infection periods and to spray the plants before infection occurs. As has already been indicated, a considerable part of the infection is brought about by the appressoria or chlamydospores. The germ tube from an appressorium having penetrated the epidermis of the host is apparently beyond the reach of injury by a fungicide; and this infection, though it may remain dormant more or less indefinitely under certain conditions, may also develop and cause serious injury under certain other conditions. The time and method of infection apparently varies in many cases, depending upon conditions which favor the production, dissemination, and germination of the spores.

# SUMMARY.

- (1) Most cultivated fruits and many other economic plants are attacked and seriously injured by fungous parasites of the genus Glomerella.
- (2) These fungi pass through three stages in the course of their complete development and produce three kinds of spores—conidia, ascospores, and chlamydospores or appressoria. Until recently the two principal spore forms, conidia and ascospores, have been described and treated as distinct organisms.
- (3) The conidial stage is most frequently observed and described, and is usually referred to one or the other of the form genera Gloeosporium and Colletotrichum. About 500 so-called species of conidial forms probably belonging to Glomerella are recorded.
- (4) The genetic connection of the conidial with the ascogenous stage was first definitely proven with cultures in 1898 by Atkinson, working with Glomerella (Gloeosporium) cingulata (Stonem.) S. and v. S. found on privet. Since that date the life-history forms, races, and species of the organism from several other host plants has been recorded by Clinton, Stoneman, Edgerton, Sheldon, Koorders, and the present writers.
- (5) The life histories of forms from 36 different host plants have been determined and recorded here. In 17 cases they were produced in pure cultures and in the other 19 cases they developed on the host either in a moist chamber or under natural conditions. In 31 cases they were first reported by the writers.

(6) In most of the forms studied neither morphological nor physiological differences sufficient for the segregation of species have been found. All the material from the 36 hosts is referred to three species of Glomerella, G. cingulata, which occurs on 34 of the hosts, G. gossypii on one, and G. lindemuthianum on one.

(7) Glomerella cingulata is exceedingly variable in all its characters so far as they have been studied. The cause of this variability is not yet clear. No constant or definite relation has been established between the cultural and other environmental conditions and the

most important variations observed.

(8) The fungus is found to be present in many cases in apparently normal and healthy foliage, fruits, and sometimes in the stems of its hosts, as shown by its development and fructification on such portions of plants after they have been thoroughly washed in a corresive-sublimate solution which has been shown to kill not only ascospores and conidia but also the chlamydospores or appressoria of the fungus. The chlamydospores or appressoria evidently send a germ tube through the epidermis of the host as shown by Hasselbring, and this remains in a quiescent condition until opportunity for further development occurs.

(9) Inoculation experiments with fruits have shown that most of the forms from different hosts will produce the characteristic Glomerella rot on fruits of other hosts. It is also shown that there is great variability in the virility of different races or strains of the fungus from the same host. In one experiment races from the lemon, grape, and fig produced more serious cases of bitter-rot of apple than a race of the fungus derived from apples. These facts are of great importance in connection with the selection and production of

disease-resistant varieties of plants.

- (10) The production or nonproduction of the perithecial stage of Glomerella appears to be a fairly well-fixed hereditary race character. Where a race of the fungus has been obtained by repeated trial of spores from different sources and races which develops both conidial and ascogenous fructifications in cultures it continues to produce them for many generations. An ascogenous race from Persea was grown for 23 generations from conidia. The last generation produced perithecia about as abundantly as the first. No evidence has been obtained to indicate that the production of perithecia is controlled by any of the ordinary conditions of nutriment or environment.
- (11) Glomerella is a parasite which has apparently developed special features, the most important of which is its method of infection by means of chlamydospores, or appressoria, and its ability to remain in a dormant or quiescent condition until the host plant becomes weakened or injured in some way or until specially favorable

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conditions for the growth of the fungus occur. In many cases the fungus never develops further until the infected portion of the host dies. The development of the fungus in seeds as in the case of cotton and bean is also evidently a special feature of Glomerella to insure its passing the winter and reaching the new crop. In nature the perithecial form, as in the case of many other pyrenomycetes, develops normally only after the death of the host tissues.

(12) The experiments of Scott, Rolfs, and others have shown that the diseases of apples and citrus fruits caused by Glomerella can be satisfactorily controlled by spraying with Bordeaux mixture. It is probable that this method can be successfully used in the prevention of the diseases of other plants caused by this fungus. The selection of fungus-free seed is also an effective method of reducing loss from disease, as shown by Barre for cotton and Whetzel for beans. Eradication and destruction of dead and diseased parts of infected plants are also important. The selection and breeding of resistant varieties may also be practicable in some cases, as indicated by the work of Bain and Essary with clover.



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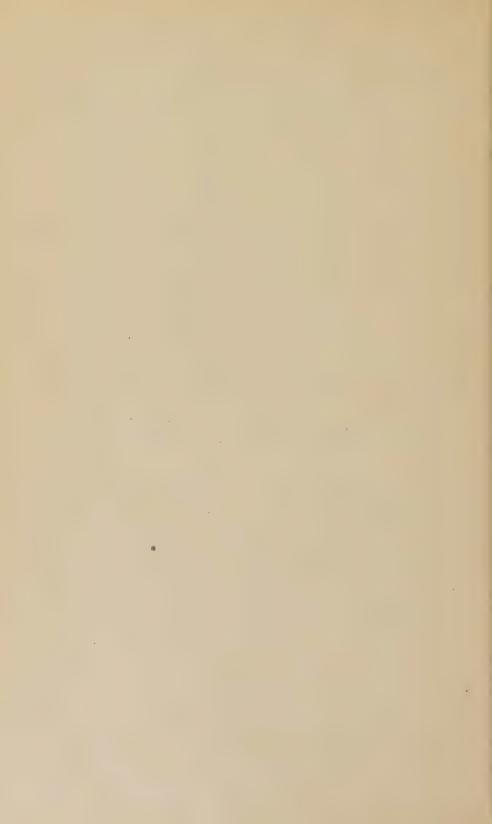
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## EXPLANATION OF PLATES.

[Plates I, II, and III are from photomicrographs made by Mr. Lon A. Hawkins, of the Office of Fruit-Disease Investigations. All asci are magnified 450 diameters and the separate ascospores 700 diameters.]

Plate I. Figs. 1 to 15.—Photomicrographs of asci and ascospores of Glomerella, from various hosts. Fig. 1.-From grape. An ascus from a glycerin mount stained in eosin, from dried corn-meal culture 325d. Fig. 1a.—From grape. Ascospores from a fresh glycerin mount, unstained, from corn-meal agar culture 1373b. Fig. 2.—From grape. An ascus from a fresh glycerin mount stained in gentian violet, from a corn-meal agar culture 1238h. Fig. 2a.—From grape. Ascospores from same culture as figure 2. Fig. 3.—From apple. An ascus from a fresh glycerin mount stained in eosin, from a dried specimen on fruit. Fig. 3a.—From apple. Ascospores from same source as figure 3. Fig. 4.—From cranberry. An ascus from an old mount, unstained, from culture 756 from skin and pulp of New Jersey berry. Fig. 4a.—From cranberry. Ascospores from same mount as figure 4. Fig. 5.—From black raspberry. An ascus from a 2-year-old formalin mount, unstained, from plate culture from canes from Shelbyville, Tenn. Fig. 5a.— Ascospores from the same mount as figure 5. Fig. 6.—From pomelo. An ascus from a formalin mount 2 years old, unstained, from a leaf from a greenhouse of the Department of Agriculture, kept in a moist chamber. Fig. 6a.—Ascospores from the same mount as figure 6. Fig. 7.—From lemon. An ascus from a mercuric chlorid mount 1 year old, unstained, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 7a.—Ascospores from the same mount as figure 7. Fig. 8.—From orange. An ascus from a glycerin mount, stained with eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 8a.—Ascospores from the same mount as figure 8. Fig. 9.—From guava. An ascus from a fresh glycerin mount from dried specimen, unstained, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 9a.—Ascospores from a fresh glycerin mount from a dried specimen, stained in eosin, from a leaf from the department greenhouse, kept in a moist chamber. Fig. 10.— From avocado. An ascus from a mercuric chlorid mount 1 year old, unstained, from leaves from the Department greenhouse, kept in a moist chamber. Fig. 10a.—Ascospores from the same mount as figure 10. Fig. 11.—From loquat. ascus from a glycerin mount, stained with eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 11a.—Ascospores from the same mount as figure 11. Fig. 12.-From cotton. An ascus from an old slide, unstained, from a culture. Fig. 12a.—Ascospores from the same slide as figure 12. Fig. 13.—From bean. An ascus from an old formalin mount, unstained, from cornmeal culture 485a, from bean pods from Takoma Park, Md. Fig. 13a.—Ascospores from corn-meal culture 484 from same source as figure 13. Fig. 14.— From coffee. An ascus from a fresh glycerin mount from a dried specimen, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 14a.—Ascospores from a fresh glycerin mount, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 15.— From camellia. An ascus from a fresh glycerin mount from a dried specimen, unstained, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 15a.—Ascospores from a fresh glycerin mount from a dried specimen, unstained, from a leaf from the Department greenhouse, kept in a moist chamber.

Plate II, Figs. 16 to 29.—Photomicrographs of asci and ascospores of Glomerella from various hosts. Fig. 16.-From tea. An ascus from a fresh glycerin mount, stained in eosin, from leaves from the Department greenhouse, kept in a moist chamber. Fig. 16a.—Ascospores from an unstained glycerin mount from the same material as figure 16. Fig. 17.—From privet. An ascus from a 2-year-old formalin mount, unstained, from twigs from Digby, Nova Scotia. Fig. 17a .-Ascospores from same mount as figure 17. Fig. 18.—From rubber plant. An ascus from a glycerin mount from fresh specimen, stained, from leaves from the Department greenhouse, kept in a moist chamber. Fig. 18a.—Ascospores from a glycerin mount, stained in eosin, from the same source as figure 18. Fig. 19.— From Ficus longifolia. An ascus from a 1-year-old formalin mount, unstained, from leaves from the Department greenhouse, kept in a moist chamber. Fig. 19a.—Ascospores from same mount as figure 19.—Figure 20.—From honey locust. An ascus from an old mount, probably formalin, unstained, from corn-meal culture from leaves from the grounds of the Department of Agriculture. Fig. 20a.—Ascospores from same mount as figure 20. Fig. 21.—From spiral flag (Costus speciosa). An ascus from a fresh glycerin mount from dried specimen, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 21a.—Ascospores from a 2-year-old formalin mount, unstained, from the same specimen as figure 21.—Fig. 22.—From Curculigo sp. An ascus from a formalin mount, 1 year old, slightly stained in methyl blue, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 22a.—Ascospores from the same mount as figure 22. Fig. 23.—From ebony (Brya). An ascus from a glycerin mount from a fresh specimen, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 23a.—Ascospores from the same mount as figure 23. Fig. 24.—From chocolate (Theobroma cacao L.) An ascus from a glycerin mount from fresh material, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 24a.—Ascospores from the same mount as figure 24. Fig. 25.—From Pitcairnia. An ascus from a fresh glycerin mount from dried specimen, stained in eosin, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 25a.—Ascospores from a glycerin mount, stained in eosin, from the same material as figure 25. Fig. 26.— From palm (Hedyscepe sp.). An ascus from an old mount, probably in formalin, unstained, from a leaf from the Department greenhouse. Fig. 26a.—Ascospores from a fresh glycerin mount, stained with eosin, from a leaf from the Department greenhouse. Fig. 27.—From ginkgo. An ascus from an old mount, probably in formalin, unstained, from a leaf from the grounds of the Department of Agriculture. Fig. 27a.—Ascospores from the same mount as figure 27. Fig. 28.— From Caryota. An ascus from a mercuric chlorid mount, I year old, unstained, from a leaf from the Department greenhouse, kept in a moist chamber. Fig. 28a.-Ascospores from the same slide as figure 28. Fig. 29.—From fig. An ascus from a fresh glycerin mount, unstained, from corn-meal agar culture 1283d from conidia on fruit from Norfolk, Va. Fig. 29a.—Ascospores from the same mount as figure 29.

Plate III. Glomerella cingulata.—Three series of asci from different hosts, showing the wide range of variation observed. Photomicrographs from fresh glycerin mounts. Figs. 30 to 34.—From grape. All from cultures of the same origin. Fig. 30.—An ascus 101 μ long, from corn-meal agar slant culture 1373b from Vienna, Va. Fig. 31.—An ascus 87 μ long, from the same culture. Fig. 32.—An ascus 77.5 μ long, from the same culture. Fig. 33.—An ascus 70.5 μ long, from another tube from the same source. Fig. 34.—An ascus 54.5 μ long, from corn-meal agar culture 1373b. Figs. 35 to 39.—Glomerella cingulata from apple. Fig. 35.—An ascus 108 μ long, from corn-meal agar slant culture 1348k, from conidia on Willow Twig apple from Vienna, Va. Fig. 36.—An ascus 89.5 μ long, from the same culture as

figure 35. Fig. 37.—An ascus 83  $\mu$  long, from same cultures as figures 35 and 36. Fig. 38.—An ascus 77  $\mu$  long, from same culture as figures 35, 36, and 37. Fig. 39.—An ascus 55  $\mu$  long, from corn-meal agar slant culture from apple leaves from Winchester, Va.—Figs. 40 to 44.—Glomerella cingulata from fig. Fig. 40.—An ascus 110  $\mu$  long, from corn-meal agar slant culture 1283d, from conidia on fruit from Norfolk, Va. Fig. 41.—An ascus 92.5  $\mu$  long, from same culture as figure 40. Fig. 42.—An ascus 85.5  $\mu$  long, from corn-meal agar slant 1283a, from same source as 1283d. Fig. 43.—An ascus 63.5  $\mu$  long, from corn-meal agar slant 1283e, from same source as 1283a. Fig. 44.—An ascus 56  $\mu$  long, from same culture as figure 43.

Plate IV. Pomelo leaves showing development in moist chamber of numerous colonies of Glomerella cingulata on apparently healthy leaves for 9 days. The lower leaf 1 year younger than the upper. The colonies originated chiefly from the midrib.

Plate V. Glomerella cingulata on pomelo leaves. Figure 1 shows development of numerous scattered colonies on apparently healthy leaves in moist chamber, evidently rising from separate points of infection. Figure 2 shows small leaf 1 year younger taken from the same plant and treated in the same manner. No fungus developed, as the leaf was apparently not infected.

Plate VI. Glomerella cingulata on 2 orange leaves, showing the development of the fungus on apparently healthy leaves in a moist chamber and the localization of the colonies which evidently originated from separate points of infection. The rubber plant leaf at the right shows the development of the fungus from the petiole along the midrib. Acervuli first developed on the petiole, which has been removed.

Plate VII. Glomerella lindemuthianum from bean. Six corn-meal agar cultures started from conidia from a single acervulus, showing the uniform character of cultures of this species and the characteristic dark mycelium usually producing acervuli only.

Plate VIII. Glomerella cingulata from Persea. Ascospore generation 2, tube a, showing separate perithecia thickly covering the surface of the medium. Conidial generation 13, tubes b and c, showing chiefly acervuli. Conidial generation 14, tubes b and c, originated from generation 13, b and c, respectively, are strikingly different. Of generation 14, b shows chiefly acervuli and conidia and c chiefly perithecia, which are arranged in masses along the line where the spores were planted.

Plate IX. Glomerella cingulata from Persea. Conidial generation 9, tube a, shows numerous scattered perithecia and a few acervuli near the bottom. Conidial generation 16, tubes b and c, from which conidial generation 17, tubes b and c, were made, produced almost entirely acervuli. Of generation 17, tube b produced almost entirely perithecia, while c was practically identical with generation 16, tube c, showing chiefly acervuli.

Plate X. Glomerella cingulata from Persea. Conidial generation 17 produced from 16, tube b, which was almost identical with tube 4. These cultures showed rather regular intergradations from tube 1, which chiefly produced perithecia, to tube 7, which produced acervuli almost entirely. Tubes all the same age.

Plate XI. Glomerella cingulata from Persea. Conidial generation 17, tube b, showing mostly perithecia, and six subcultures from the same. All the cultures except the one at the extreme right showed a great predominance of acervuli, with but few perithecia, showing a reversion to the form in generation 16, tube b.

Plate XII. Glomerella cingulata from Persea. Showing striking variations. Conidial generation 17. Tubes d, e, f, and g, all originated from generation 16, tube b. Compare Plate IX. Of generation 17, tubes d, e, and f show mostly perithecia; g has perithecia at the bottom of the culture and acervuli above. Of conidial generation 19, tube b shows mostly acervuli, while of generation 20, tube b produced mostly perithecia.

Plate XIII. Glomerella cingulata from Persea. Seven tubes from conidial generation 17, derived from generation 16, tube c, which produced chiefly acervuli. These cultures all produced, chiefly perithecia which were arranged in dense, scattered black masses, instead of being separate and evenly distributed, as in most cases of the preceding generations. Compare Plate IX.

Plate XIV. Glomerella cingulata from Persea. Two plates poured from conidial generation 9, tube a. Conidia were numerous at first in both plates, but were scattered, and no conspicuous acervuli seen. Only one colony, a, produced distinct and conspicuous acervuli. The rest of the colonies were chiefly perithecia.

Compare Plate IX, tube 9 a.

Plate XV. Glomerella cingulata from Persea. Plates 10 days old made from crushed perithecia and ascospores. The irregular, scattered, large, dark bodies, a, are colonies of acervuli. The other colonies are chiefly perithecia. This strain of the fungus originated from a single conidium. A greater development of perithecia is observed along lines of contact between the conidial and perithecial colonies.

Plate XVI. Willow Twig apples inoculated with conidia of Glomerella cingulata from 4 different hosts. Figures 1 and 2 inoculated with conidia from an apple; figures 3 and 4, from a lemon; figures 5 and 6, from grapes; figures 7 and 8, from a fig. The strain of the fungus from the apple showed less virulence in this instance than that obtained from other hosts. All were inoculated at the same time, in the same manner, and kept under the same conditions.

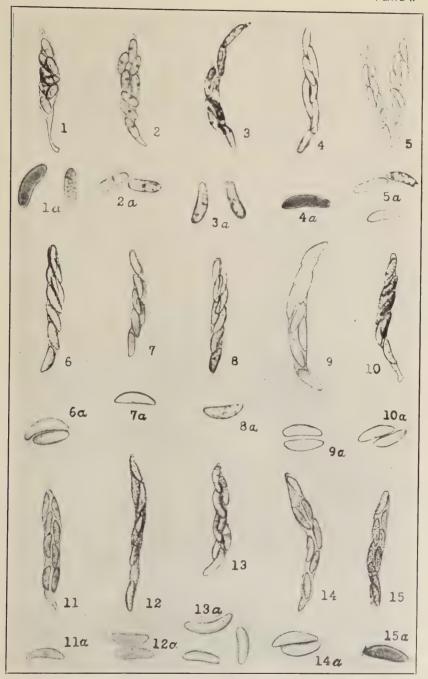
Plate XVII. Eight figs 13 days after inoculation with conidia of *Glomerella cingulata* obtained from a rubber plant. The four upper fruits were inoculated by puncture; the four lower by surface application. All except two of those inoculated on the surface developed the common Glomerella rot of the fig.

Plate XVIII. Watermelon inoculated with conidia of Glomerella cingulata obtained from guava. The decayed area is practically covered with large acervuli.

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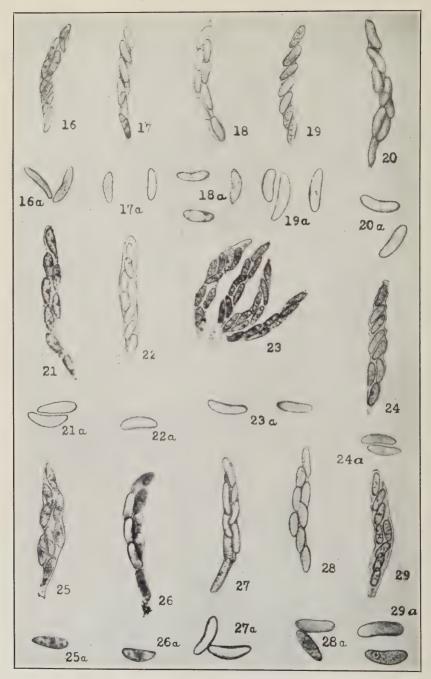
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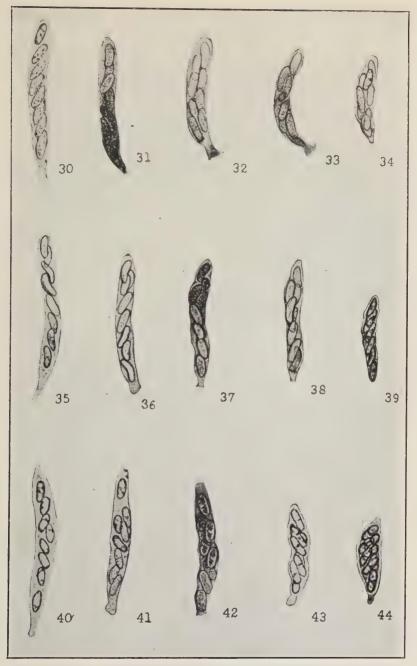
GLOMERELLA ASCI AND ASCOSPORES FROM VARIOUS HOSTS.

Figs. 1 and 2 from grape; 3, apple; 4, cranberry; 5, black raspberry; 6, pomelo; 7, lemon; 8, orange; 9, guava; 10, avocado; 11, loquat; 12, cotton; 13, bean; 14, coffee; 15, camellia. Asci  $\times$  450, separate ascospores  $\times$  700 diameters,



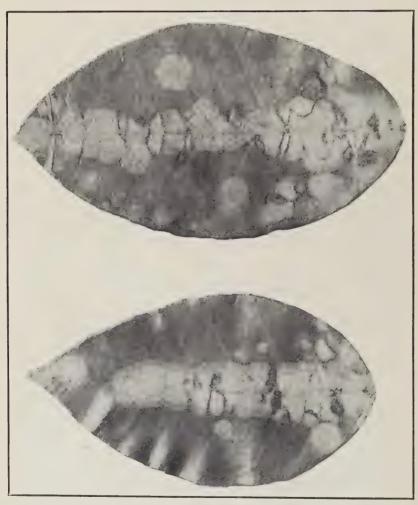
GLOMERELLA CINGULATA ASCI AND ASCOSPORES FROM DIFFERENT HOSTS.

Fig. 16 from tea; 17, privet; 18, rubber plant; 19, Ficus longifolia; 20, honey locust; 21, spiral flag; 22, Curculigo; 23, ebony; 24, chocolate; 25, Pitcairnia; 26, palm; 27, Ginkgo; 28, Caryota; 29, fig. Asci × 450, separate ascospores × 700 diameters.

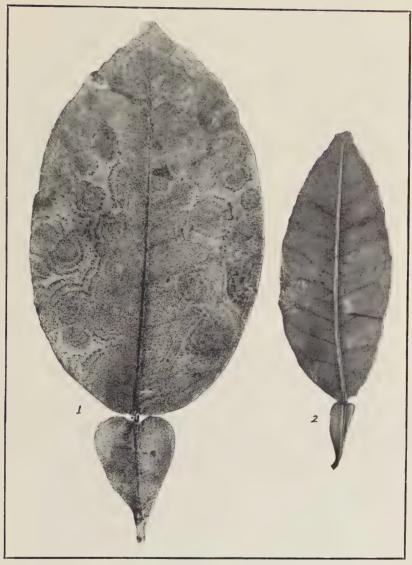


GLOMERELLA CINGULATA ASCI FROM THREE DIFFERENT HOSTS, SHOWING THE RANGE OF VARIATION IN EACH.

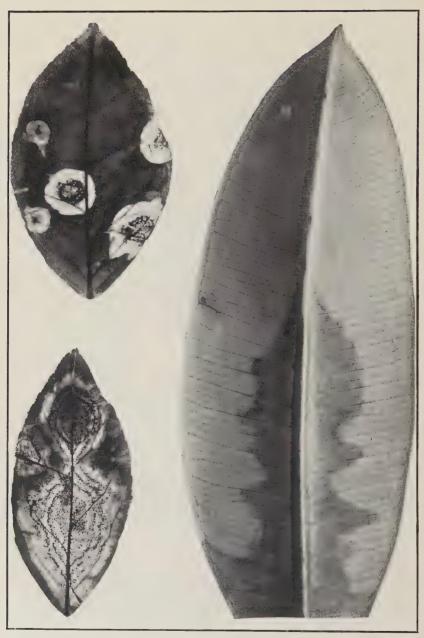
Figs. 30 to 34 from grape; 35 to 39, apple; 40 to 44, fig. All  $\times$  450 diameters.



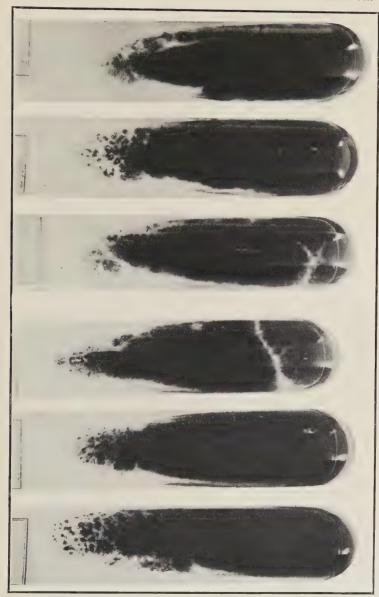
POMELO LEAVES, SHOWING DEVELOPMENT IN MOIST CHAMBER OF NUMEROUS COLONIES OF GLOMERELLA CINGULATA ON APPARENTLY HEALTHY LEAVES. ONE YEAR'S DIFFERENCE IN THE AGE OF THE LEAVES. THE COLONIES MOSTLY ORIGINATED FROM THE MIDRIB.



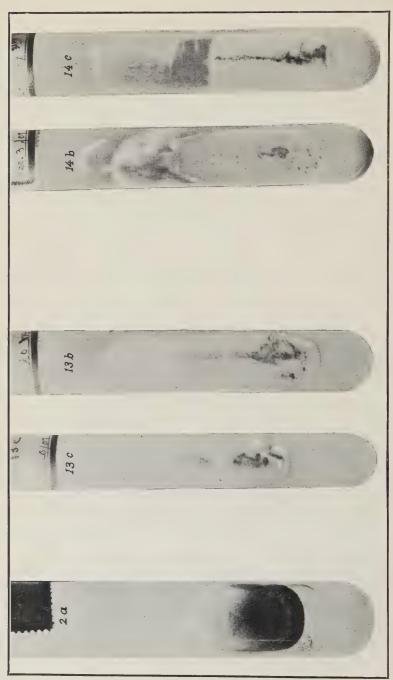
Pomelo Leaves, Showing (1) Development of Colonies of Glomerella cinculata in Moist Chamber and Apparent Localization of Numerous Points of Infection; (2) the Small Leaf One Year Younger, from Same Plant Treated in the Same Manner, Apparently not Infected.



GLOMERELLA CINGULATA ON TWO ORANGE LEAVES, SHOWING DEVELOPMENT OF THE FUNGUS ON APPARENTLY HEALTHY LEAVES IN A MOIST CHAMBER AND LOCALIZATION OF THE COLONIES. THE RUBBER PLANT LEAF AT THE RIGHT SHOWS DEVELOPMENT OF THE FUNGUS PROCEEDING FROM THE PETIOLE ALONG THE MIDRIB.

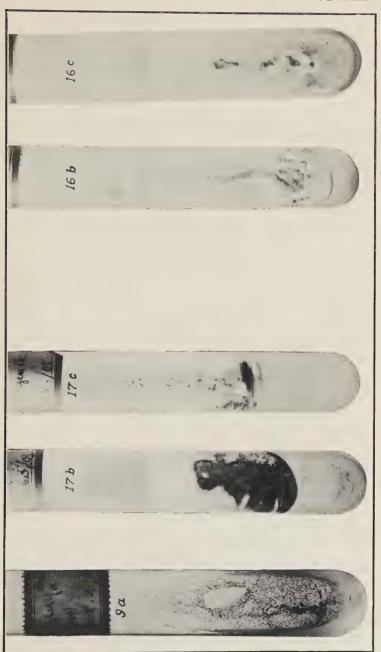


GLOMERELLA LINDEMUTHIANUM CULTURES FROM BEAN. SIX TUBES FROM CONIDIA FROM A SINGLE ACERVULUS, SHOWING THE UNIFORM CHARACTER OF THE CULTURES AND THE DARK MYCELIA PRODUCING ACERVULI ONLY.



GLOMERELLA CINGULATA CULTURES FROM PERSEA-1.

Ascospore generation 2, tube a, showing the black perithecia thickly covering the surface. Conidial generation 13, tubes b and c, chiefly acervult; and conidial generation 14, tubes b and c, b shows chiefly acervult and conidia and c chiefly perithecia growing in masses along the line of incentation.



GLOMERELLA CINGULATA CULTURES FROM PERSEA—II.

Conidial generation 9, tube  $a_i$ , showing numerous perithecia and a few acervuli near the bottom. Conidial generations 16 and 17, tubes b and  $c_i$ . Sixteen b produced almost entirely acervuli, 17 b almost entirely perithecia, while 17  $c_i$  like 16  $c_i$  is chiefly acervuli.

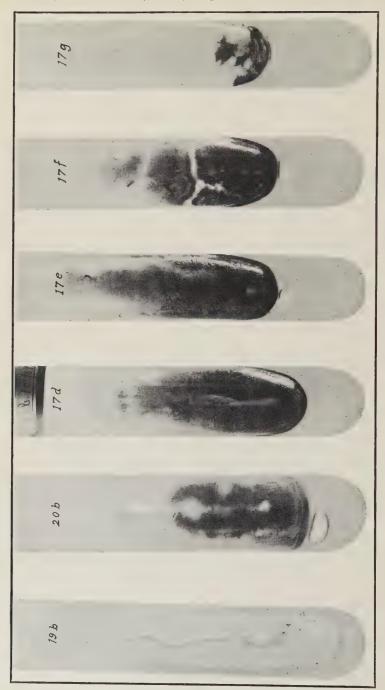


Conidial generation 17, consisting of subculture from generation 16, tube b, showing rather regular intergradations from tube 1 containing chiefly acervuli. Compare Pl. XI. GLOMERELLA CINGULATA CULTURES FROM PERSEA-III,

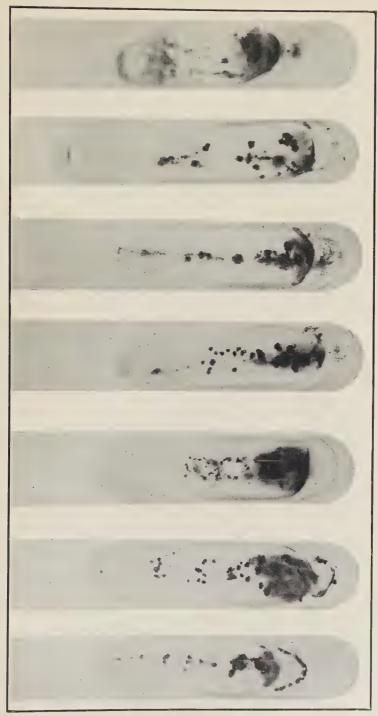


GLOMERELLA CINGULATA CULTURES FROM PERSEA-IV.

Conidial generation 17, tube b, showing mostly perithecia, and six subcultures from the same. These tubes, except the one at the extreme right, showed a great predominance of acervuli with few perithecia, as in 16 b. Compare Pl. X.



Conidial generation 17, tubes d, c, f, and g, all from 16 b. Compare Plate IX. Tubes 17 d, c, and f, mostly perithecia. g, perithecia below and acervuli above: conidial generation 19, tube b, mostly acervuli; generation 29, tube b, mostly perithecia. GLOMERELLA CINGULATA CULTURES FROM PERSEA-V, SHOWING STRIKING VARIATIONS.



Seven tubes of confidial generation 17, from generation 16, tube c, which produced chiefly accrvuli. These tubes show chiefly perithecia in dark masses. Compare Pl. IX. GLOMERELLA CINGULATA CULTURES FROM PERSEA-VI, SHOWING VARIATIONS.



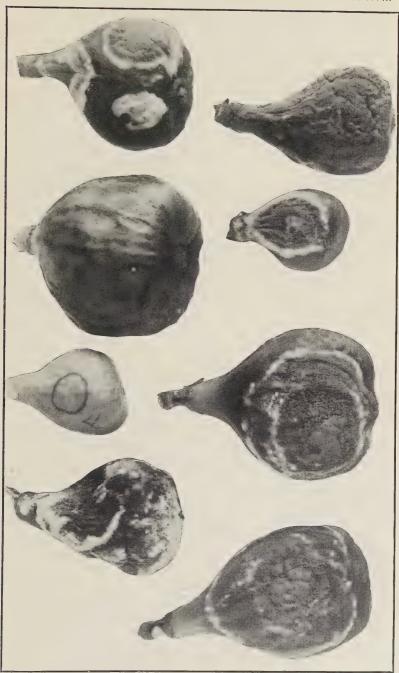
Two plates from conidial generation 9, tube a. Only one distinct colony, a, produced large accrvuli, the rest chiefly perithecia. Compare Plate IX, tube 9 a. GLOMERELLA CINGULATA CULTURES FROM PERSEA-VII.



The irregular, scattered, large, dark spots, a, are colonies of acervuli, the others perithecia. Note the greater development of perithecia at the lines of contact between these colonies and the perithecial colonies. GLOMERELLA CINGULATA CULTURES FROM PERSEA-VIII. PLATES 10 DAYS OLD FROM CRUSHED PERITHECIA AND ASCOSPORES.



WILLOW TWIG APPLES INOCULATED WITH CONIDIA OF GLOMERELLA CINGULATA FROM DIFFERENT HOSTS. Figs. 1 and 2, from apple; 3 and 4, from lemon; 5 and 6, from grape; 7 and 8, from fig.



The four upper fruits were inoculated by puncture, the four lower by surface application. All except two of those inoculated on the surface developed rot. FIGS 13 DAYS AFTER INOCULATION WITH CONIDIA OF GLOMERALLA CINGULATA FROM A RUBBER PLANT.



Watermelon Inoculated with Conidia of Glomerella cingulata from Guava. The Decayed Area is Practically Covered with Large Acervuli.

